

THE EXTRAHEPATIC BILIARY TRACT IN THE GUINEA-PIG¹

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THIRTEEN FIGURES

For the last several years investigations on the physiology of the liver and the biliary tract of common laboratory animals have been in progress at the Institute of Experimental Medicine. Recently, in studying the emptying of the gall bladder, following the administration of the Boyden fat meal, we observed that the gall bladder emptied by the contraction of its own intrinsic musculature; this was also recognized by Whitaker and others. It was found to be true not only for the laboratory animals, but for fishes, amphibia, and birds as well.

In studying (with Mann(3)) the emptying gall bladder in dogs, cats and guinea-pigs we observed that the emptying process would not take place in animals under ether or urethane anesthesia; this apparently explains why investigations on anesthetized animals fail to record definite emptying of the vesicle. Accordingly, any direct observations that are made on the emptying gall bladders must be made by the use of some local anesthetic.

It was found that the biliary tract in the guinea-pig was excellently suited for investigating the mechanism involved in emptying the gall bladder. In contrast to the rabbit and the dog, in which the gall bladder is firmly bound to the liver substance (this may be the reason why the vesicle in these species does not empty completely), the vesicle in the guinea-

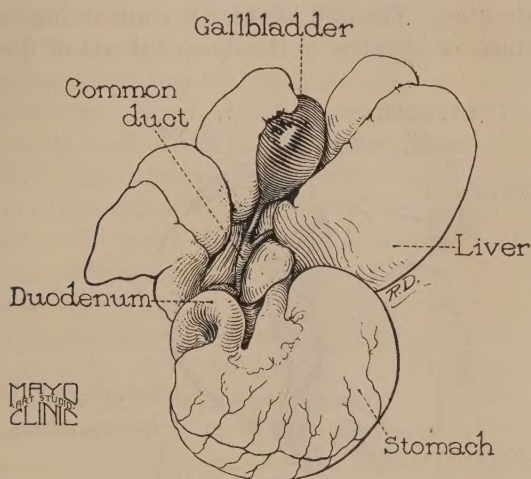
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pig hangs relatively free from the liver. There is, however, a single membrane which suspends the gall bladder from the central lobe of the liver; but this may be readily severed and the entire extrahepatic biliary tract thus satisfactorily exposed. In their earlier studies on the comparative physiology of the biliary tract, Mann, Brimhall, and Foster observed and portrayed the gall bladder in the guinea-pig, together with the peculiar bile-evacuating mechanism at the lower end of the common duct. No adequate description of this peculiar mechanism was given at that time; but with the recent observations on the contractile mechanism within the gall bladder it has seemed advisable to describe in some detail certain of the anatomic structures and physiologic processes associated with the biliary tract in this animal, particularly since such a mechanism as exists at the lower end of the common bile duct of the guinea-pig has not been observed in any other animal.

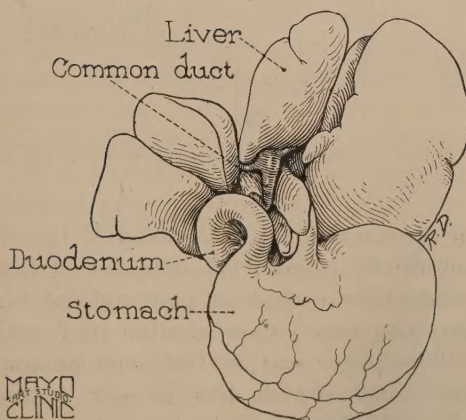
In general, three main lobes comprise the liver in the guinea-pig (fig. 1). These lobes are subdivided in various ways, by clefts of varying depths, into smaller lobes, so that a constant liver pattern as such does not exist. In general, the left lobe is usually the largest and extends ventral to the cardia and fundus of the stomach, while the central lobe is smaller, usually cleft, and suspends the gall bladder from its ventral surface. The right lobe is usually more elongated than the other two and is invariably subdivided into two or three smaller lobes which frequently envelop the anterior pole of the right kidney.

Although there are occasional variations, the extrahepatic biliary tract in this animal comprises the gall bladder, cystic duct, two main hepatic ducts, and the common duct. A gall bladder is usually present, although recently a case was noted in which it was absent (fig. 2). In this animal the common duct was dilated in much the same manner as is characteristically found after removal of the gall bladder. Besides the two hepatic ducts, a smaller hepatic duct is frequently found which drains that portion of the central lobe

supporting the gall bladder. It empties into the cystic duct just below the neck of the gall bladder. The common bile duct is variable both in length and in size and empties into the duodenum from 5 to 6 mm. caudad to the pylorus. The



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Fig. 1 Liver and biliary tract of a normal guinea-pig.

Fig. 2 Liver and biliary tract of a guinea-pig without a gall bladder.

size of the common duct observed in any particular guinea-pig is dependent on the physiologic status of the biliary apparatus at the moment of death. Figure 3 shows a biliary tract with a common duct fully three times the size of that shown in figure 1. Two factors have determined the extent of this distention. The gall bladder is contracting, and, since the sphincteric mechanism at the duodenal end of the common

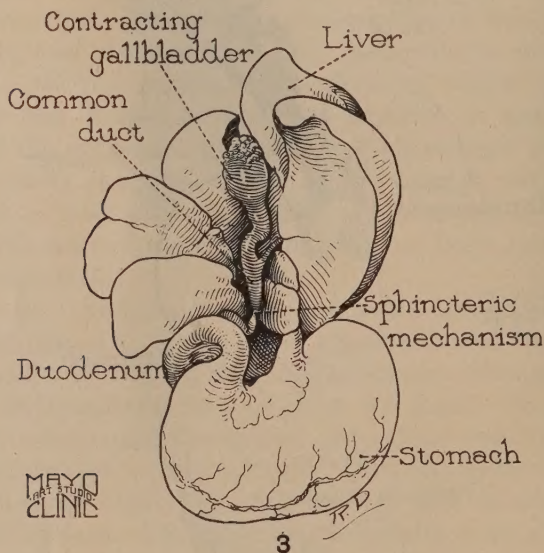


Fig. 3 Liver and biliary tract of a guinea-pig in which the gall bladder is partially contracted.

bile duct is in contraction, the elasticity of the choledochus permits of such dilation under the influence of the increased pressure. In no other rodent or mammal, as far as I am aware, is there a common duct similar in function to the guinea-pig's. The lower end of this duct expands into an elongated oval pouch which lies closely applied to the duodenum and is held firmly to it by the peritoneal membrane. From the inferior surface of this pouch near its caudal margin a narrow duct passes directly through the wall of the

duodenum to empty into the lumen of the gastro-intestinal tract. This dilated portion of the common duct bears a significant relationship to the discharge of bile, the physiologic significance of which will be considered in a later section.

The extrahepatic biliary mechanism of the guinea-pig is exceedingly delicate. When the gall bladder is more or less completely distended, as in the fasting animal, its wall is nearly transparent and the contained bile is invariably clear and translucent. Four layers may be quite definitely delineated in the wall of the gall bladder: the mucosa, the fibro-elastic layer, the muscularis, and the serosa (fig. 4). The mucosa is composed of a single layer of columnar cells, quite closely set with nuclei situated centrally or basally. Large folds of the mucosa, projecting into the lumen of the vesicle, are usually absent in the gall bladder during fasting, while mucous glands, such as those described by Sudler for the pig and the ox, are only occasionally seen. With differential stains (Mallory and van Gieson) the layer immediately below the mucosa is shown to be largely made up of elastic tissue. There is a considerable interlacing of elastic fibers with the muscle layer, but certainly the preponderance of connective tissue lies immediately below the mucosa. As regards this particular study, perhaps the most significant layer of the wall of the gall bladder is the muscularis. Certain it is that this mechanism is sufficiently extensive to effect the evacuation of the gall bladder by its own contraction. Recently, Chiray and Pavel have shown that the muscularis of the gall bladder of the guinea-pig is composed of two layers of smooth-muscle fibers. The outer layer, adherent to the delicate serosa, comprises bundles of fibers of varying size which take a course largely at right angles to the major axis of the gall bladder. These bundles are not parallel, but diverge widely, forming thereby an extensive network of more or less oval mesh. Immediately internal to this outer layer the muscle fibers course in a more longitudinal direction, but likewise form an extensive interlacing network. Fibers coursing in a diagonal direction may also be identified. In



Fig. 4 Section of the gall bladder of a guinea-pig in average distention.
× 350.

Fig. 5 Liver of a guinea-pig, showing the gall bladder completely contracted.

the newborn guinea-pig muscle fibers are abundant, but the elastic-tissue elements remain more or less confined to a distinct layer just beneath the mucosa; in the adult a certain mingling of these tissues has occurred.

This description by Chiray and Pavel of the arrangement of the muscle bundles in the wall of the gall bladder in the guinea-pig is well borne out by the manner in which the gall bladder contracts. Higgins and Mann, in their studies on the emptying of the exposed gall bladder, noted that the vesicle did not empty by contraction waves. Rather, there appeared to be independent areas of contraction, so that the vesicle gradually changes its shape from completely spherical to pronounced angular. Thus there is not a gradual even reduction in the extent of the tunic wall, but by the more or less independent contraction of these various areas, small herniations of varying size are produced irregularly over the surface of the vesicle (fig. 3). These small herniations, made possible by the peculiar disposition of the smooth-muscle bundles, usually appear first over the fundus and then gradually increase in number and extent until they involve practically the entire surface of the vesicle, so that gall bladders have frequently been observed in a state of complete contraction (fig. 5).

A simple demonstration may readily convince one of the contractile force of this muscle layer. If the gall bladder of a guinea-pig is carefully exposed under local anesthesia, the picture of these small herniations may be produced by either stroking the vesicle with a blunt instrument or by stimulating it electrically. Figure 3 shows the biliary tract of a guinea-pig in which the gall bladder is in a partial state of contraction, as evidenced by the small herniations which appear over its fundus. This particular status of the vesicular wall was induced electrically, but frequently, following the administration of a fat meal, the gall bladder has been observed in a similar contracting state. The response of the muscularis layer to the various stimuli is not constant, for one may not predict the speed with which the gall bladder

may empty either in a normal intact animal or in one in which the vesicle is in some way stimulated. The actual amount of smooth-muscle bundles is, no doubt, variable, and with this the variable physiologic tone at the time of observation serves to produce inconstancy in results.

In a large series of guinea-pigs which were killed for other purposes, observations were made of the time required to evacuate the gall bladder, following a brief stimulation by an induction current. In each case when the abdomen was opened the duodenum was suspended at a point well above the level of the gall bladder, so that gravity could not be a factor in the flow of bile from the vesicle. The time for complete evacuation of the gall bladder in this series of animals ranged from three to seven minutes. In some animals the response of the gall bladder to the current was immediate, while in others the action was much more prolonged. Coupled with the general tone of the wall of the gall bladder in this response is the physiologic status of the sphincter at the duodenal end of the common bile duct. Relaxation of the sphincter is accompanied by rapid evacuation of the bladder, while contraction of the sphincter offers much opposition to the flow of bile. It seems certain that the peculiar mechanism at the lower end of the common bile duct has not eliminated the necessity for the independent contraction of the gall bladder, for when the common duct is severed near the duodenum and connected to a pressure manometer, it is found that the gall bladder readily contracts against a pressure even as high as 70 to 80 mm. of water.

When the gall bladder of the guinea-pig begins to contract, the mucosa responds by the formation of numerous small folds which extend into the lumen of the vesicle (fig. 6). The cells comprising the mucosa become even more columnar, as though they were more compressed laterally; and in many instances the layer appears to be composed of two or three cells, as though they were forced one over the other. A considerable amount of elastic tissue extends out into these folds, but the muscularis remains definitely a part of the



Fig. 6 Section of a gall bladder of a guinea-pig, partially contracted. $\times 20$.

Fig. 7 Section of a portion of the wall of a gall bladder of a guinea-pig in advanced contraction. Note the thickness of the muscularis and the extent of the folds of the mucosa. $\times 50$.

deeper wall and retains its usual position. It necessarily becomes thicker as the process of contraction goes on. As further reduction goes on and the capacity of the vesicle is greatly decreased, these mucosal folds become markedly increased. They branch frequently, anastomose with other folds, and thereby form large irregular spaces in the lumen of the vesicle (fig. 7). Furthermore, large crypts arise within the elastic layer, formed by outpocketings of the mucosa into the outer layers of the vesicle. These crypts, however, communicate by small openings with the gradually decreasing lumen of the gall bladder.

These observations, both anatomic and physiologic, lead to the conclusion that the gall bladder in the guinea-pig has sufficient muscle tissue to effect complete contraction. Considerable elastic tissue is present, but it does not exceed in amount the smooth-muscle tissue. The formation of these small herniations, the appearance of the mucosa under varying conditions of emptying, and the increase in relative thickness of the muscularis itself lead to the conclusion that the gall bladder empties its bile by its own contraction under proper conditions of stimulation.

In the guinea-pig the relation of the common duct to the duodenum differs from that of any other known animal. Instead of coursing diagonally through the duodenal wall, as in many laboratory animals, the lower end of the choledochus in the guinea-pig, as already mentioned, expands into a large oval pouch, from the caudal end of which a small duct passes directly through the duodenal wall. Thus the bile, in passing through the lower portion of the choledochus into the intestine, pursues a course somewhat resembling the letter S (fig. 1). As one watches the evacuation of the gall bladder experimentally, it is interesting to note the activity of this small pouch at the duodenal end of the common duct. This organ is highly muscular and waves of contraction pass in a cephalocaudal direction over it, thus forcing the contained bile into the duodenum. These waves of contraction seem to take origin from a level on the choledochus just above the

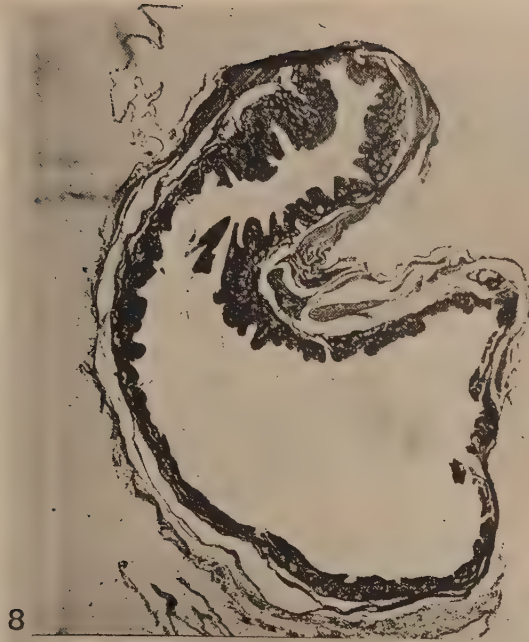
expanded pouch, where a definite constriction of the common duct rhythmically manifests itself. This point of constriction is clearly shown in figure 3 and is designated as a sphincteric mechanism. Histologic studies of the common duct at this point reveal a rather abrupt increase in the amount of smooth muscle. Studies of sections of the choledochus above this level stained to differentiate the connective tissue from the muscle fibers (van Gieson) show that the connective-tissue content is large, but that smooth-muscle bundles are only occasionally present and interspersed largely with elastic tissue (fig. 8). There are numerous outpocketings of the mucosa into the deeper connective-tissue layer which form the saccular dilatations so frequent among common ducts in other animals. At the site of this constriction in the choledochus, however, where the contraction process is first definitely evident, there is an abrupt increase in the amount of muscle tissue (fig. 9). The fibers in this region pursue a more or less circular course. Here, too, there is an abundance of elastic tissue below the muscularis, but in addition numerous connective-tissue bundles interlace with the smooth-muscle fibers. Gradually from this level the amount of muscle tissue in this portion of the choledochus increases as it approaches the duodenum, and where the duct continues into the expanded pouch the muscularis of the duct continues into that of the pouch. In this region the muscle layers are exceedingly abundant, and differential staining reveals that there are at least three layers of muscle fibers present (figs. 10 and 11). These, however, are not readily differentiated, for there is considerable interlacing of fibers, which together effect the peculiar pulsating movement observed in the mechanism. From the inferior surface of the pouch and near its caudal margin where the small channel passes through the intestinal wall into the gastro-intestinal lumen, there are numerous smooth-muscle fibers which are definitely circular and extend out to the tip of the papilla, where lies the orifice of the common duct (fig. 12).

Here, then, is a highly muscular mechanism, adapted to the distal end of the common duct related in some way to the evacuation of bile. There is no doubt but that it should be designated as a sphincter, although it does differ significantly from those biliary tracts with which we commonly associate duodenal sphincters. In order to determine the degree of contractile force that could be induced within this mechanism, a cannula was inserted into the common duct directed toward the duodenum. The duct was then carefully dissected away from the intestinal wall, and the small bile channel which courses through the duodenal wall was severed just below the expanded pouch. The end of the inserted cannula was then connected with a pressure bottle within which warm Ringer's solution was maintained at a constant body temperature. By means of a drop recorder and signal magnets, the rate of flow of the solution through the duct could be taken. The distal expanded pouch of the common duct was then placed on an electrode and stimulated briefly. The response was immediate. The contraction of the muscle bundles in the pouch immediately closed the duct, and the solution could not pass through. In from ten to twelve seconds following stimulation, the mechanism had relaxed and the flow of the solution gradually returned to the frequency shown before the period of stimulation. Figure 13 is a record taken of this particular observation. After repeated or prolonged stimulation, the muscle layers fail to relax and constant retention is effected. This simple experiment is conclusive evidence that this unusual apparatus at the distal end of the common duct has a pressure resistance sufficiently great to regulate the discharge of bile into the duodenum.

Although this evacuating mechanism is probably affected by peristaltic activity of the duodenum, it seems, neverthe-

Fig. 8 Cross-section of the common duct of the guinea-pig above the region of the sphincteric mechanism. $\times 35$.

Fig. 9 Cross-section of the common duct of the guinea-pig through the upper portion of the sphincteric mechanism. $\times 50$.



Figures 8 and 9



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Fig. 10 Cross-section of the lower portion of the common duct and distal pouch of the guinea-pig just before their junction. A portion of the duodenum is also shown. $\times 35$.

Fig. 11 Cross-section of the distal pouch of the common duct of the guinea-pig in its position on the duodenal wall. Two folds of the duodenum are included. $\times 35$.

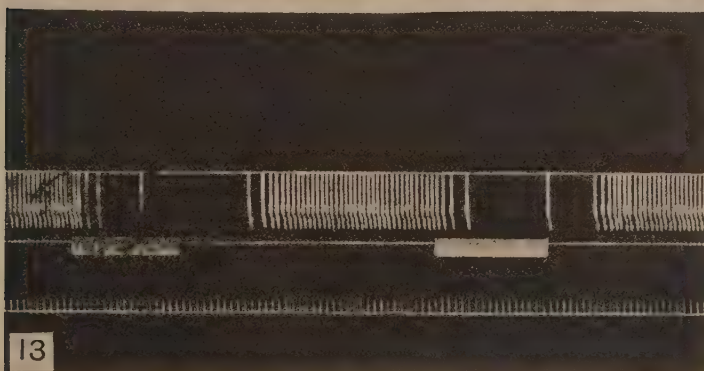
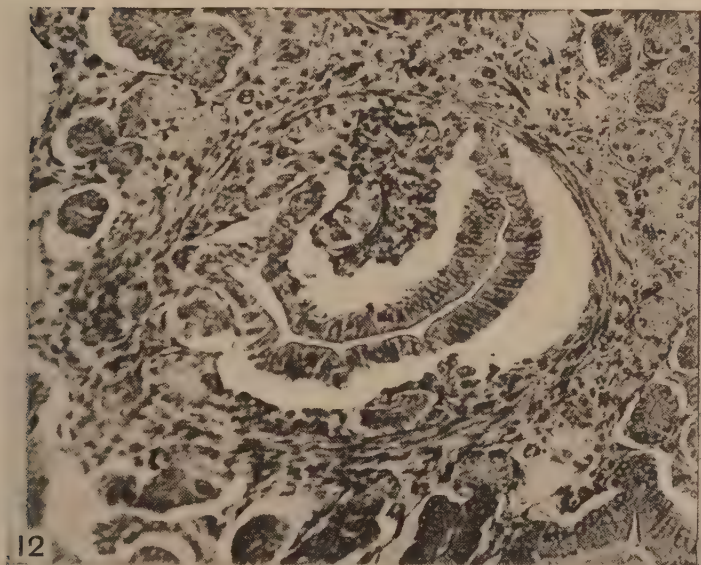


Fig. 12 Cross-section of the papilla of the common duct of the guinea-pig near the orifice into the gastro-intestinal tract. $\times 200$. Note the circular muscle fibers of the common duct which extend even to the tip of the papilla.

Fig. 13 Photograph of a record of the drop recorder, showing the flow of warm Ringer's solution through an isolated common duct of a guinea-pig, before, during, and after brief electrical stimulation.

less, certain that it is not dependent on such intestinal movements. I have often observed the active pulsations of this contractile pouch when there were no visible signs of peristalsis. Furthermore, the greater part of the musculature of the mechanism is anatomically independent of the muscle layer of the duodenum. It is probably true that a peristaltic wave may set up certain coordinate movements in this sphincteric mechanism, but I feel that the action of the latter may be entirely independent of that of the intestine. When the duodenum is severed along a line opposite to the side of the papilla, the bile is seen to leave the orifice in gentle spurts. This force, not great, is induced by the pulsations of this dilated portion of the common duct. In these observations there was no evidence that peristalsis was effective in forcing the bile into the duodenum, for certainly there were no visible signs of such motility.

The function of this peculiar arrangement must in some way be associated with the discharge of bile from the gall bladder. The contraction of the mechanism may entirely inhibit the flow of bile into the duodenum, and in such cases I have frequently observed the dilatation of the upper one-half of the common duct to thrice its normal size, and wide distention of the cystic duct. It seems certain that the flow of bile from the gall bladder, while in some way related to the activity of the lower end of the common bile duct, is a function of the muscle layer of the wall of the gall bladder. It is not suction that draws the bile from the vesicle, for the gall bladder may evacuate without regard for this mechanism. If the common duct is severed at a level just above this expanded portion and connected by a cannula with a pressure manometer, it is found that the vesicle will empty and that it will empty against a pressure as high as 70 to 80 mm. of water. The sequence of events in emptying the gall bladder is somewhat as follows. Gall bladder bile is forced from the gall bladder by the force of its own contractile mechanism in the manner above described. This bile passes through the common duct into the dilated pouch

which lies adjacent to the duodenum. Then, by the contraction of the band of circular muscle fibers near the lower end of the common duct, which keeps the bile from passing backward into the biliary tract, and by the waves of contraction which pass over the distal pouch, this portion of bile is forced into the duodenum. The sphincteric mechanism then relaxes, and contraction of the gall bladder forces a new portion of bile into the pouch. Then, by the contraction of the sphincter, together with the wave-like movements of the distal pouch, a second spurt of bile enters the duodenum. The egress of bile then appears to be dependent on a series of muscular contractions involving in sequence the gall bladder, the sphincteric mechanism, and the dilated pouch at the distal end of the choledochus.

The guinea-pig is one of the few animals in which the ductus choledochus empties into the gastro-intestinal tract so near to the pylorus. In gophers, rats, and mice, as well as cats and dogs, the intestinal orifice of the common duct is considerably caudal to the stomach. Then, too, the guinea-pig is one of the very few animals in which the common duct passes directly through the intestinal wall. This anatomic feature probably has some direct relationship to the peculiar dilation of the lower end of the choledochus. Here is an elongated oval pouch, parallel in its greatest dimension to the gastro-intestinal tract. In that sense it is comparable to the intra-duodenal portion of the common duct in other animals. It may be that this mechanism is not only concerned with the evacuation of bile and the filling of the gall bladder, but that it insures the emptying of this portion of the duct and prevents regurgitation from the duodenum.

SUMMARY

Microscopic study of the biliary apparatus shows that smooth-muscle fibers are abundant in the gall bladder, relatively sparse in the hepatic ducts and the upper portion of the common bile duct, but exceedingly abundant in the lower portion of the common duct and its expanded pouch. Elastic

fibers are present in the gall bladder, but less numerous than the smooth-muscle fibers.

The evacuation of the gall bladder is affected by the contraction of the smooth-muscle layer in the wall of the gall bladder. Reduction in the size of the gall bladder first appears in the fundus where small herniations arise by the contraction of separate areas in the muscularis. These herniations increase in size and finally involve the entire gall bladder, and thus together completely evacuate the vesicle.

The discharge of bile into the duodenum is the result of the contraction of the gall bladder together with the contraction of the expanded pouch at the distal end of the common bile duct. When the gall bladder contracts, bile is forced into the cystic and the common ducts and thence into the lower expanded pouch. The sphincteric mechanism just above this pouch then contracts, and by the forceful contraction of the wall of the pouch itself the contained bile is forced into the duodenum. The sphincteric mechanism prevents the bile from passing back into the common duct. Gall-bladder bile thus passes into the duodenum by the successive contractions of the gall bladder, the sphincteric mechanism, and the pouch at the lower end of the common bile duct.

The extrahepatic biliary tract of the guinea-pig differs from that of any other known animal in the presence of a peculiar bile-evacuating mechanism at the lower end of the common bile duct. This portion of the biliary tract is highly muscular, and in the discharge of bile from the gall bladder, rhythmic pulsations pass over it in a cephalocaudal direction.

LITERATURE CITED

- 1 BOYDEN, E. A. 1925 The effect of natural foods on the distention of the gall bladder, with a note on the change in pattern of the mucosa as it passes from distention to collapse. *Anat. Rec.*, vol. 30, pp. 333-364.
- 2 CHIRAY, M., AND PAVEL, I. 1925 La contractilité de la vésicule biliaire. *Jour. de physiol. et de path. gen.*, T. 28, pp. 105-111.
- 3 HIGGINS, G. M., AND MANN, F. C. 1926 Observations on the emptying of the gall bladder. *Am. Jour. Physiol.*, vol. 78, pp. 339-347.
- 4 MANN, F. C., BRIMHALL, S. D., AND FOSTER, J. P. 1920 The extrahepatic biliary tract in common domestic and laboratory animals. *Anat. Rec.*, vol. 18, pp. 47-66.
- 5 SUDLER, M. T. 1901 The architecture of the gall bladder. *Johns Hopkins Hosp. Rep.*, vol. 12, pp. 126-129.
- 6 WHITAKER, L. R. 1926 The mechanism of the gall bladder. *Am. Jour. Physiol.*, vol. 78, pp. 411-436.

A NOTE ON A RARE CONGENITAL PELVIC KIDNEY

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TWO FIGURES

The condition here recorded was noticed in the regular course of anatomical dissection. At first glance it appeared to be a pelvic tumor. However, the removal of the peritoneum covering its anterior surface clearly revealed a somewhat atypical kidney in a midpelvic position.

This displaced left kidney was about normal in size for the given individual and was evidently a functioning organ. The right kidney was slightly hypertrophied.

The pelvic kidney was composed of three lobes, each with an artery of supply. The right lobe and the upper left lobe were each supplied by an artery arising from the crotch of the aortic bifurcation. The lower left lobe was supplied by a large artery taking origin from the left internal iliac artery. The venous return was as follows: The right and the upper left lobes were drained by veins emptying into the vena cava, while the lower left lobe and a small part of the lower pole of the right lobe drained through a large renal vein into the left internal iliac vein. The ureter was short, only 4 inches in length, but was a functionally normal duct arising from four components which represented the split-up renal pelvis, as is seen in figure 1. One stem of the renal pelvis entered from the upper left lobe, one from the lower left lobe, while two stems entered the single ureter from the large right lobe of the kidney. The ureter ran the usual course through the lower pelvic region and into the bladder.

The right suprarenal gland was normal and placed immediately above the normal right kidney. The left suprarenal was in the normal abdominal position, and its venous return emptied into the vena cava at the level for the usual renal vein of the left side. The left spermatic vein, in the absence of the normally placed left renal vein, drained into

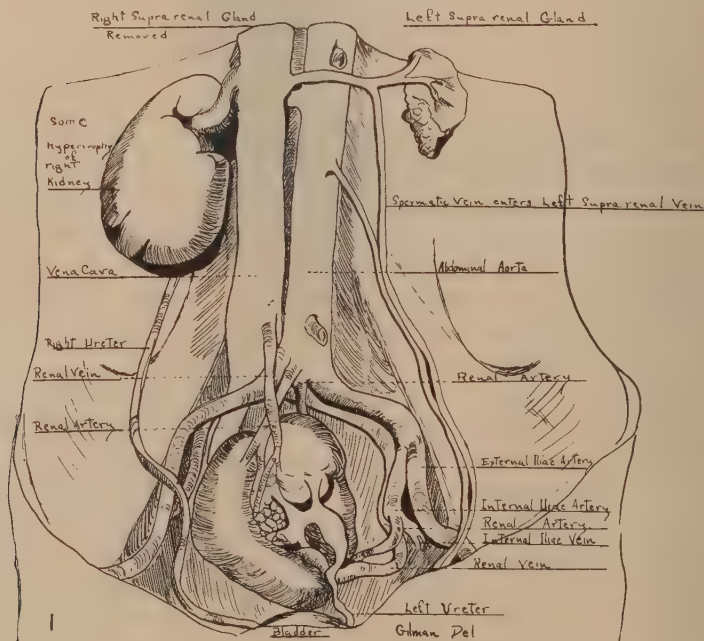


Figure 1

the left suprarenal vein. The arrangement on the right side was typically normal.

The posterior surfaces of the three lobes of the pelvic kidney were so molded as to be closely fitted over the promontory of the sacrum. The anterior surface of this kidney was thus immediately posterior to the lower end of the pelvic colon and the rectum.

The subject in which this kidney occurred was a rather well-nourished male, and the available hospital records gave no indication that this condition had ever caused trouble.

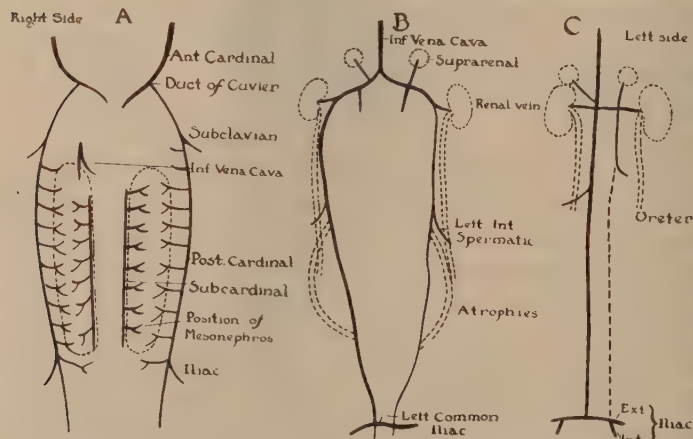
Such a condition is not difficult to explain from the standpoint of early human development. A figure in Keibel and Mall, *Human Embryology*, volume 2, page 833, shows the relation of the ureter bud to the umbilical artery and also the relation of the lower end of the primary excretory duct, the wolffian duct, from which will arise the collecting system of the permanent kidney, the metanephros. Since the metanephric blastema lies in close relation to the umbilical artery and also to the aorta, and since the excretory blastema is segmental in structure, one expects and does find that the arterial supply will be derived from the segmental vessel of the vicinity.

Hence to these three metanephric anlagen which lie in relation to the umbilical artery and to the aorta, arterial branches of supply will grow. At birth the umbilical artery atrophies and there remains the hypogastric or internal iliac artery with its branch of supply to the lowermost lobe of this misplaced pelvic kidney, while the upper two lobes derive vessels from the aorta. The determination of the permanency of this anlage as a kidney structure depended upon the growth of the ureter bud and its communication with these lower segments of the renal blastema to form a normal renal pelvis and collecting tubules united to the excretory renal blastema, the cortical part of the kidney. The low position of the metanephric anlage would attract, by biochemotaxis, the ureter bud and cause their union.

Figure 2, reading from left to right, diagrams the progress of venous development. It shows the change in venous drainage from that of the mesonephros to that of the permanent kidney, the metanephros. It indicates the venous return from the suprarenal, and the position in development of that structure. Among the several hundred cases of developmentally displaced kidneys, not one of these cases describes the adrenal gland as being out of the normal developmental

position, and, of course, there is no reason from development why it should follow the kidney.

A reference to the figure in Keibel and Mall, volume 2, page 696, after a wax reconstruction by Mall, shows the condition of the venous return of the pelvis. This diagram shows numerous tributaries which might become of vast importance in the presence of such a condition as a pelvic kidney. A venous return may anastomose with, or drain into, any vein of the vicinity during development, and that segment of the



2 Diagrams of the Stages in the development of the Inferior Vena Cava

Figure 2

nephric blastema which lies in the proximity of the vena cava will drain by a vein into the vena cava, and a similar communication with the internal iliac vein may readily be established.

From ancient to modern literature the record of kidney abnormalities abounds. Vesalius, Eustachius, Baubin, and Haller have described the absence of one kidney. Keen classifies kidney abnormalities as follows: Abnormalities in number, abnormalities in position, abnormalities in form, and congenital and acquired abnormalities. Keibel and Mall

classify them as: Abnormalities in number, abnormalities in number of ureters, and abnormalities in position.

W. Roberts, in an article on anomalies of position, form, and number of kidneys (*System of Med.*, Reynolds, London, 1879), states that in abnormalities of position the adrenal remains in the usual position. He describes a kidney on the brim of the pelvis in a female in which parturition was delayed, but successfully terminated. He further believed that usually only one kidney is involved. In his cases, as well as in ours, the sigmoid was on the right side coursing in front and to the right of the pelvic kidney.

Dougal Bissell described a kidney on the brim of the pelvis—an acquired condition—with a blood supply from higher up, and he replaced this kidney at operation.

Congenital absence of both kidneys has been described, but is very rare, while congenital absence of one kidney is also rare, but more frequent. Horseshoe kidneys are comparatively common, but true congenital pelvic kidneys, as here reported, seem to be the really rare condition.

Keen states that pelvic kidneys have been observed with a vessel of supply from the common iliac artery, and another has been observed with a supply from the internal iliac artery, with a venous return in each case to the corresponding vein. But this condition, with its arterial supply of two stems from the abdominal aorta, and a third supply from the internal iliac artery, with a venous return by two single veins, one into the vena cava and the other into the internal iliac vein—a truly functioning kidney, well-shaped, perhaps just a little undersized, pressed firmly against the promontory of the sacrum—seems to be unique.

LITERATURE CITED

- KEEN Surgery.
Index Medicus.
Index catalogue of the library of the Surgeon-General's office, U. S. Army,
series I, II.
BISSELL, DOUGAL 1910 Pelvic kidney. Gynecological Transactions.
Zur pathologischen Anatomie der Bildungsanomalien in uropoetischen System.
ROBERTS, W. 1879 Anomalies of position, form and number of kidneys. Sys-
tem of Med., Reynolds, London, vol. 5, pp. 639-649.
GOSSELIN 1869 L'Union Med., p. 115
GOULD AND PYLE Anomalies and curiosities of medicine.
BUGBEE AND LOSEE The clinical significance of congenital anomalies of the
kidney and ureter.
JENKINSON Vertebrate embryology.
BAILEY AND MILLER A text-book of embryology.
BARDELEBEN The anatomy of man.
KOLLMANN'S Atlas.

DUPLICATION OF PANCREATIC BLADDER AND ACCESSORY PANCREAS IN THE CAT

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ONE TEXT FIGURE AND FOUR PLATES

A pancreatic bladder and two fragments of accessory pancreas were discovered in a cat during experiments on intestinal movements.

The pancreatic bladder lay to the right of the gall bladder (fig. 1) and attached to it except at the fundus. Its appearance was white, while the gall bladder had the characteristic greenish-blue color of this organ. Both vesicles were covered by a common fold of peritoneum, but there was no communication between their respective cavities.

The bile and pancreatic ducts opened normally into the duodenum. The duct of the pancreatic bladder, about 5 cm. in length, had a sharp kink just below its vesicular origin. From the level of this kink the duct diverged to the right in its course, and finally became attached to the body of the pancreas about 2 cm. to the right of the emergence of the duodenal portion of the pancreatic duct. The surface area of pancreatic tissue immediately surrounding the termination of the bladder duct was slightly raised and felt hard to the touch.

The pancreatic vesicle was very turgid, and an attempt was made to empty its contents gently by squeezing with a pair of forceps. The only displacement observed was confined to the bladder and its duct, both of which bulged irregularly. This result suggested that the bladder had no open communication with the pancreatic duct. An incision was next made in the duct of the bladder about 6 mm. from its

pancreatic termination. Approximately 4 cc. of a watery fluid, faintly yellow in color, were recovered. The fluid was then examined under the microscope. Numerous small concretions were found, but no cholesterol crystals or greenish pigment could be detected. In appearance this fluid bore no resemblance to bile. One accessory pancreas, measuring 7 mm. in length and 5 mm. in width, lay at the tail end of the major pancreas. It was attached to the pancreas by means of two or three fibrous strands, one of which later proved to be a duct. The other accessory pancreas was found between the submucous and muscular layer of the duodenum, about 4 cm. caudad to the ampulla of Vater.

HISTOLOGICAL EXAMINATION

The pancreatic bladder and its duct

The idea of making a histological examination of this particular anomaly came as an afterthought, prompted partly by investigations made on a previous case (Bean and Dreyer, '27) and partly by the fact that, except for Boyden ('25), scanty reference has been made to the histology of pancreatic bladders in most publications on the subject. Unfortunately, the decision to fix the material for a histological examination was made without any serious intention of publication; therefore, the tissue did not receive proper treatment in many respects. For example, the bladder and its duct should not have been pressed with forceps to force out the contained fluid, and the whole structure should have been thoroughly washed out with normal saline previous to fixation. As a result of rough handling and imperfect penetration of the fixing fluid, many of the final preparations were imperfect. Furthermore, the vesicle hardened very rapidly in the higher grades of alcohol, became rather brittle, and subsequently sectioned poorly.

Zenker's fluid was chosen as the fixing agent for all the tissue saved for histological study. The entire vesicle and about two-thirds of its duct was fixed in toto. The remainder of the duct proximal to the normal pancreas, together with a

section of pancreas which enclosed the duct, was also fixed. Both accessory pancreases as well as a section of the normal pancreas were likewise prepared. For purposes of comparison, a portion of the gall bladder and a piece of the hepatic duct was also killed in Zenker's fluid.

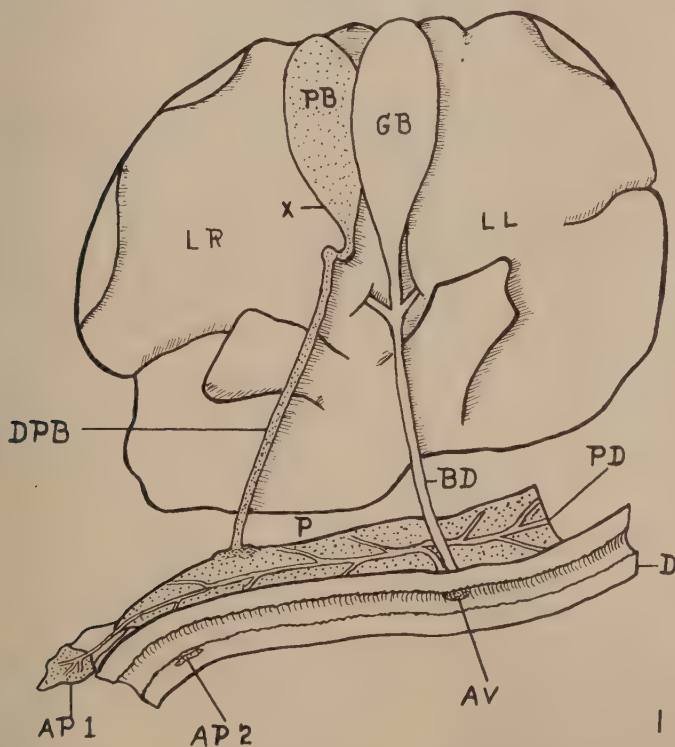


Fig. 1 Diagram showing the gross anatomical relationships of the pancreatic bladder and accessory pancreases. *AP 1*, accessory pancreas attached by fibrous strands and a duct to the tail of the major pancreas; *AP 2*, accessory pancreas embedded in the wall of the duodenum; *AV*, ampulla of Vater; *BD*, bile duct; *D*, duodenum; *DPB*, duct of the pancreatic bladder; *GB*, gall bladder; *LL*, left lobe of the liver; *LR*, right lobe of the liver; *P*, pancreas; *PB*, pancreatic bladder; *PD*, pancreatic duct. The approximate location of a secondary vesicle within the wall of the pancreatic bladder is indicated by *X*.

The paraffin method of infiltration was used, and most of the sections were cut 10μ in thickness. Several different staining methods were tried to bring out the essential histological differentiation of the tissue. Haematoxylin and eosin, Van Giesen's stain, Wiegert's elastic-tissue stain, and Heidenhain's iron haematoxylin proved to be the most valuable. All photomicrographs were made from iron-alum haematoxylin preparations.

The pancreatic vesicle is made up of four coats—an inner mucous, a submucous, a muscular, and an outer fibrous tunic.

The fibrous tunic consists of both white and yellow fibers, with a few isolated smooth-muscle elements arranged longitudinally. Both myelinated and non-myelinated nerve fibers may be distinguished, and, at the junction between the fibrous and muscular coats, ganglion cells and nerve plexuses are present. Large blood vessels course through the loosely arranged fibrous tissue.

The muscular coat, made up of circularly disposed smooth-muscle fibers, is poorly and unevenly developed. Bundles of fibro-elastic tissue frequently break the continuity of the muscle layer and separate the contractile elements of this coat.

The submucous tunic is composed of rather loose areolar tissue with many elastic fibers and large numbers of elongate or oval connective-tissue cells. The blood vessels are small but numerous. In some areas the circular muscle is only separated from the mucous membrane by an extremely narrow zone of submucous tissue. The inner surface of this tunic is often thrown into low folds which bear some resemblance to the valvulae conniventes of the intestine. Large, coiled submucous glands are sparsely distributed throughout the submucosa, and they communicate with the free surface of the mucous membrane. Ganglion cells are commonly found along the boundary between the muscular and submucous tissue (fig. 5).

The mucous coat is made up of a single layer of columnar epithelium, resting on a thin basement membrane. The tunica

propria projects inward toward the lumen of the vesicle in the form of tall cylindrical papillae. These papillae, many of which are branched, are richly supplied with blood vessels which form definite capillary loops at the distal extremities (figs. 6 and 7). In some cases clear-cut openings (fig. 8) suggest the presence of large central lymph sinuses. These papillae occur profusely throughout the lining of the vesicle. The columnar epithelial cells possess large oval nuclei which lie at the basal portion, while the cytoplasm is distinctly granular. Unfortunately, since the secretions within the vesicle were not washed out thoroughly at the time of fixation, histological differentiation of the free border of the epithelium is impossible. For the same reason, it is difficult to decide whether or not many true goblet cells are present. Certainly, there are often definite breaks in the free border of the epithelium, while the basal portion shows absolute continuity, that is, the nuclear alignment is unbroken. Such a condition is most easily explained by assuming that true goblet cells are very common. In addition, transverse sections of the papillae or villus-like structures often show cells in which the nucleus is decreased in size and crowded down to the very basal portion of the cell, while the non-granular cytoplasm is usually swollen and partially displaces the adjacent cellular units.

The histological structure of the duct which runs between the vesicle and the pancreas represents a continuation of the vesicular tunics. Transverse sections taken close to the vesicle more nearly resemble the vesicle in structure than sections taken farther along in its course. The villus-like projections of the mucous membrane are soon lost, and the duct takes on the general characteristics of the pancreatic ducts with occasional tubular glands along its course. One odd fact should be noted at this point. About 4 mm. from the region where the duct begins to expand into the vesicle proper, a narrow diverticulum of its mucous membrane extends parallel to the lumen of the duct and ends blindly as a small vesicular pouch (fig. 12), enclosed within the fibrous outer tunic of

the main vesicle. This secondary duct resembles the main duct histologically, but the secondary vesicle has the appearance of normal gall bladder in a moderately distended condition (figs. 11 and 13).

Reference has already been made to the fact that a portion of the duct adjacent to the pancreas and a mass of pancreatic tissue surrounding the duct were fixed in toto. This mass of pancreatic tissue, roughly pyramidal in shape, measured about 8 mm. in depth and 6 mm. in diameter. Serial sections were made of the entire block of tissue in hopes of tracing the origin of the duct. A study of these sections shows that the duct runs independently along through the capsular investment of the gland, and at no point does it communicate with the system of pancreatic ducts. It is to be regretted that a larger portion of pancreatic tissue was not preserved. One very significant point is revealed, however. In its course through the pancreatic capsule the lumen of the duct becomes smaller and at certain points nearly obliterated. Moreover, it is certain that the duct did not leave the capsule of the pancreas and enter the duodenum independently, since the preliminary examination of the liver, gall bladder, pancreas, and duodenum was made with an idea of determining this very point.

The accessory pancreases

Accessory pancreas no. 1, which lay near the tail of the main organ, has all the histological characteristics of a perfectly normal pancreas. It communicates with the major pancreas by means of a single duct which terminates in one of the main pancreatic ducts. Interveolar islets are common and normal in appearance.

Accessory pancreas no. 2 (fig. 15), which lay between the submucosa and the inner circular muscle of the duodenum, bears a close resemblance to normal pancreatic tissue. The alveoli and interveolar islets present the usual histological picture except for the absence of well-defined interlobular ducts. Moreover, this accessory gland was absolutely iso-

lated from the major pancreas, and the histological preparations reveal no communication with the mucous membrane of the duodenum.

DISCUSSION

It is unnecessary to review the various theories which have been suggested as to the cause of pancreatic bladders in the cat. The recent publications of Bremer ('23) and Boyden ('25) give an excellent résumé of the literature on the subject. Some workers have attributed this anomaly to aberrant development of the ventral pancreas, some incline toward the 'cleft gall bladder' hypothesis, while others offer as an explanation the dual nature of the liver diverticulum. Boyden's work is of special significance, and to him belongs the credit of first attacking this problem from a histological point of view. Therefore, his statement that "the wall of the pancreatic bladder is that of an hypertrophied pancreatic duct in all the specimens I have examined," should be seriously considered in all future discussions.

The pancreatic bladder described above shows many striking similarities to the bladder which Boyden ('25) has described, figured, photographed, and designated as 'Case 2.' Superficially, at least, figure 15, plate 2, of his publication might be easily substituted for figure 10, plate 3, of this paper. Likewise, his insistence on dissimilarities in structure between gall bladder and the pancreatic vesicles which he has personally examined is in perfect accord with the findings in the present study. It certainly involves an unnecessary stretch of the imagination to consider the vesicle herein described as modified or aberrant biliary tissue. On the other hand, there appear to be marked structural differences which make Boyden's conclusions on the origin of pancreatic bladders of doubtful application in this particular case.

The presence of a secondary vesicle embedded within the wall of the primary vesicle is unusual. Since the secondary vesicle is morphologically derived from the duct of the primary vesicle, one would expect it to resemble the primary

vesicle in structure. On the contrary, however, this secondary pouch conforms to the structural characteristics of normal gall bladder (figs. 11 and 14). The mucous membrane of the primary vesicle may be considered as representing the lining of an hypertrophied pancreatic duct, but does it not also resemble intestinal tissue? Undoubtedly, the form of the mucous membrane became changed upon collapse of the vesicle from its state of superdistention. It is probable that whatever the original pattern of the mucous membrane, it must have suffered considerable modification in structure due to the high pressure of the vesicular contents. Yet, sections of mucous membrane cut away from the submucous tissue, cleared, and mounted in balsam (fig. 4), show a profusion of villus-like structures which are comparable to the lining of the intestine. Longitudinal and transverse sections of these villous projections (figs. 6, 7, 8, 9), examined under high-power lenses, only bring out closer similarities between these structures and true intestinal villi. Then, too, the occurrence of submucous glands and the presence of ganglion cells corresponding to the ganglia of Meissner serve further to heighten the analogy.

The embryological investigations which have been made with the idea of explaining the origin of the pancreatic bladder represent an important step in the solution of the problem. However, any solution which is based largely on the evidence of developmental anatomy is open to certain objections. The anomaly itself is but the morphological expression of a physiological differentiation carried out in embryonic life. At the time the anomaly is examined the primary causative agents have ceased to be effective, and one can only conjecture as to their identity. It is true that the problem of the pancreatic bladder can hardly be separated from the problem of normal development of the pancreatic and biliary systems, since in either case the embryological origin may be traced to a relatively restricted area in the primitive gut tract. This implies some sort of mechanism by which cells of like potentialities become morphologically segregated. In the case of

the liver bud, such segregation may be actually demonstrated according to Weber ('03). Assuming the postulate of segregation, one is next led to admit the possibility of imperfect segregation which may ultimately result in varying degrees of structural abnormality. This hypothesis would partially explain the presence of gall bladder and intestinal tissue in a diverticulum from the pancreatic duct. It would also account for the origin of the island of pancreatic tissue embedded within the wall of the duodenum as described above, just as well as the assumption that this accessory gland was originally derived as a diverticulum from the pancreatic duct. This idea is consistent in part with Bremer's interpretation ('23), and, in addition, it allows for the possibility of cells of unlike potentialities developing *en masse* to form the type of anomaly under discussion.

But a physiological differentiation must precede morphological segregation. There exists an abundance of evidence to substantiate this claim. For example, Pressler ('11), Morrill ('19), Carey ('20), in attacking the fundamental problem of normal asymmetry, show conclusively that the factors controlling normal asymmetry are operative long before the liver bud is a morphological entity. Pressler, who was able to produce complete *situs inversus viscerum* in *Bombinator* by experimental methods, shows that direction of growth of the liver bud may be entirely changed. The mechanism of differentiation is as yet unrevealed, though experimental results prove that it may be often partially controlled or even altered. Stockard ('21) has given an exceedingly clear account of the control of differentiation through environmental factors. This success in "locating more or less definitely the critical moment of origin" of several developmental processes, including the liver and pancreas, is surely significant. His findings are in accord with those of Pressler, Morrill, Carey, and others, in that the primary causative agents are effective physiologically long before any morphological expression can be discerned.

Furthermore, assuming the importance of "development rate and structural expression" as presented in Stockard's paper, the origin of the pancreatic bladder is readily explained in terms of a developmental arrest. It is possible that peculiarities in the mechanism of implantation of the cat ovum result in a temporary deficiency of the oxygen supply. Should this oxygen deficiency become operative at, or near, the critical moment of origin of the liver bud, one would expect to find aberrant development of the pancreatic and biliary systems in the cat of common occurrence. Such an explanation is possibly no less obscure than that which is based purely on the evidence of developmental anatomy.

It is suggested that more light may be thrown on the problem of the pancreatic bladder through a combination of the following methods of approach: 1) Further investigation on the mechanism of implantation in the cat. 2) Through continued experimental work on the general problem of growth and differentiation. 3) Through histological examination of each new case. 4) Through a biochemical analysis of the contents of pancreatic vesicles. 5) By investigation of the functional activity of these anomalies.

BIBLIOGRAPHY

- BEAN, R. J., AND DREYER, N. B. 1927 A pancreatic bladder in the cat, structurally analogous to gall-bladder. *Trans. Nova Scotia Inst. Sci.*, vol. 17, part 1, p. 60.
- BELL, HOWARD H. 1922 Horizontal and vertical pancreas, in association with other developmental abnormalities. *Anat. Rec.*, vol. 23, p. 315.
- BECKWITH, CORA J. 1920 Note on a peculiar pancreatic bladder in the cat. *Anat. Rec.*, vol. 18, p. 363.
- BOYDEN, E. A. 1922 A typical pancreatic bladder developed from an accessory pancreas. *Anat. Rec.*, vol. 23, p. 195.
- 1925 The effect of natural foods on the distention of the gall-bladder, with a note on the change in pattern of the mucosa as it passes from distention to collapse. *Anat. Rec.*, vol. 30, p. 333.
- 1925 The problem of the pancreatic bladder—a critical survey of six new cases based on new histological and embryological observations. *Am. Jour. Anat.*, vol. 36, p. 151.
- 1926 The accessory gall-bladder. An embryological and comparative study of aberrant biliary vesicles occurring in man and the domestic animals. *Am. Jour. Anat.*, vol. 38, p. 177.

- BREMER, J. L. 1923 Pancreatic ducts and pancreatic bladders. *Am. Jour. Anat.*, vol. 31, p. 289.
- BROMAN, IVAR 1922 Über die Phylogenese der Gallenblase. Abstract in *Berichte u. d. ges. Physiologie und Experimentelle Pharmakologie*, Bd. 5, S. 30.
- CAREY, E. J. 1920 Studies in the dynamics of histogenesis. *Jour. Gen. Phys.*, vol. 3, p. 61.
- CHILD, C. M. 1899 Biological lectures from the Marine Biological Laboratory of Woods Hole, p. 231.
- 1915 Individuality in organisms. Chicago.
- DRESBACH, N. 1911 An instance of pancreatic bladder in the cat. *Anat. Rec.*, vol. 5, p. 365.
- DRIESCH, H. 1894 Analytische Theorie der organische Entwicklung. Leipzig.
- GAGE, S. H. 1879 The ampulla of Vater and the pancreatic ducts of the domestic cat. *Amer. Quart. Mic. Jour.*, vol. 1, p. 123.
- HEUER, G. L. 1906 The pancreatic ducts in the cat. *Johns Hopkins Hosp. Bull.*, vol. 8, p. 267.
- JACH, E. 1899 Über Duodenaldivertikel, S. 1, Inaug. Diss., Kiel.
- JACKSON, C. M. 1908 An unusual duodenal diverticulum. *Jour. Anat. and Phys.*, vol. 42, p. 219.
- JOHNSON, C. E. 1914 An additional case of pancreatic bladder in the domestic cat. *Anat. Rec.*, vol. 8, p. 267.
- LAGUESSE, E. 1905 Le pancréas. *Revue Générale d'Histologie.*, T. 1, Fasc. 4.
- LARSELL, O. 1920 Pancreatic bladders. *Anat. Rec.*, vol. 18, p. 345.
- LEWIS, F. T., AND THYNG, F. W. 1908 The regular occurrence of intestinal diverticula in embryos of the pig, rabbit, and man. *Am. Jour. Anat.*, vol. 7, p. 505.
- LEWIS, F. T. 1911 The bi-lobed form of the ventral pancreas in mammals. *Am. Jour. Anat.*, vol. 12, p. 389.
- LUDWIG, E. 1919 Zur Entwicklungsgeschichte der Leber, des Pankreas, und des Vorderdarms bei der Ente und beim Maulwurf. *Anat. Hefte*, Abt. 1, Bd. 56, S. 513.
- MANN, F. C. 1920 Accessory pancreas in the dog. *Anat. Rec.*, vol. 19, p. 263.
- 1922 An accessory pancreas in the wall of the gall-bladder of the dog. *Anat. Rec.*, vol. 23, p. 351.
- MANN, BRINHALL, AND FOSTER 1920 The extrahepatic biliary tract. *Anat. Rec.*, vol. 18, p. 47.
- MAYER, A. C. 1815 Blase für den Saft des Pankreas. *Deutsch. Arch. f. d. Physiol.*, Bd. 1.
- MILLER, W. S. 1904 Three cases of pancreatic bladder occurring in the domestic cat. *Am. Jour. Anat.*, vol. 3, p. 269.
- 1905 A pancreatic bladder in the domestic cat. *Anat. Anz.*, Bd. 27, S. 119.
- 1910 Pancreatic bladders. *Anat. Rec.*, vol. 4, p. 15.
- MORRILL, C. V. 1919 Symmetry reversal and mirror imaging in monstrous trout, and a comparison with similar conditions in human double monsters. *Anat. Rec.*, vol. 16, p. 265.
- PRESSLER, K. 1911 Beobachtungen und Versuche über den normalen und inversen Situs Viscerum et Cordis bei Anurenlarven. *Arch. f. Ent.-Mech.*, Bd. 32.

- STOCKARD, C. R. 1919 Developmental rate and the formation of embryonic structures. *Proc. Soc. Exp. Biol. and Med.*, vol. 16, p. 93.
- 1921 Developmental rate and structural expression: An experimental study of twins, 'double monsters' and single deformities, and the interaction among embryonic organs during their origin and development. *Am. Jour. Anat.*, vol. 28, p. 115.
- WEBER, A. 1903 *L'Origine des glandes annexes de l'intestin moyen chez les vertébrés*. Thèse, Nancy.

PLATE 1

EXPLANATION OF FIGURES

2 Transverse section through the pancreatic bladder. $\times 17$. *V*, villus; *G*, submucous glands.

3 Transverse section of the duct of the pancreatic bladder. $\times 53$. *DSV*, diverticulum from the main duct of the pancreatic bladder which terminates in a blind pouch embedded within the wall of the bladder. Note the hypertrophied wall of the duct.

4 Surface view of the mucous membrane of the bladder, $\times 53$, cut away with a scalpel, cleared, and mounted in balsam. The tissue was stained lightly with haematoxylin. Note the profusion of villous structures.

5 Section through the wall of the bladder, showing a nest of ganglion cells between the submucous and muscular tunic. $\times 53$.

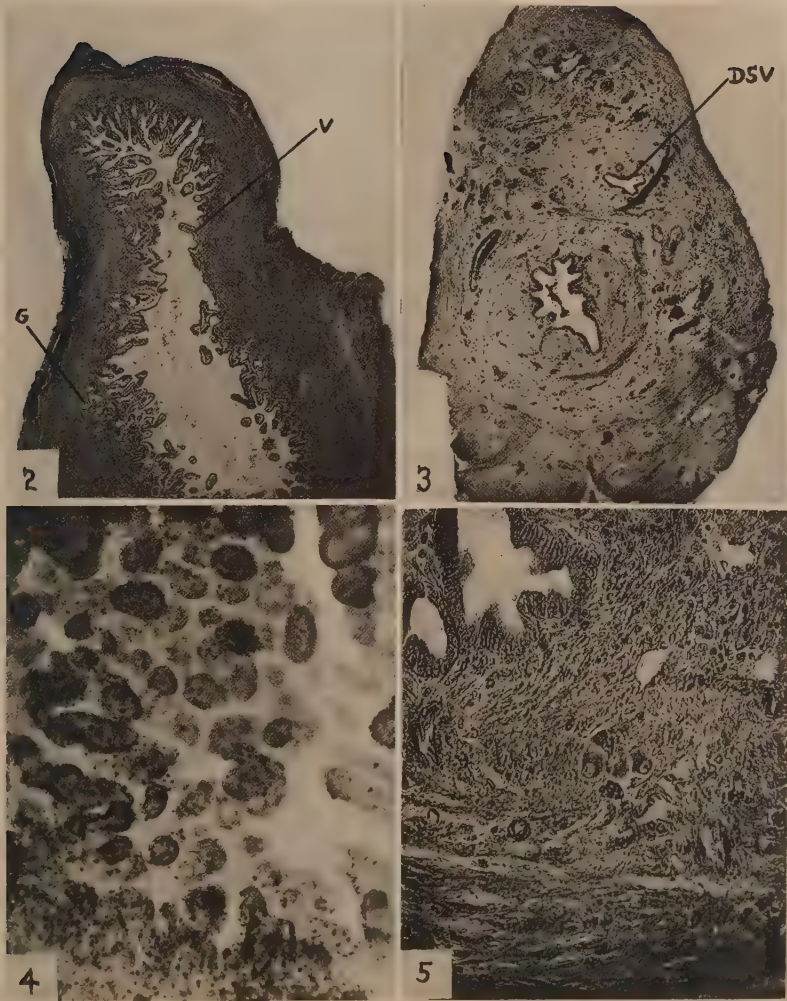


PLATE 2

EXPLANATION OF FIGURES

6 Longitudinal section through a villus, the same villus which is indicated in figure 2. $\times 225$. *CL*, capillary loop at distal extremity of the villus. Imperfect fixation is responsible for the shrinkage of the tunica propria.

7 Longitudinal section through the same villus as in figure 6. $\times 563$. Note the blood corpuscles in the capillary loop.

8 Longitudinal section through a villus. $\times 225$. *LV*, central lymph vessel, comparable to a lacteal.

9 Transverse sections of a group of three villi. $\times 225$. These same structures are visible within the vesicle at the lower right of figure 2. *G*, goblet cells.

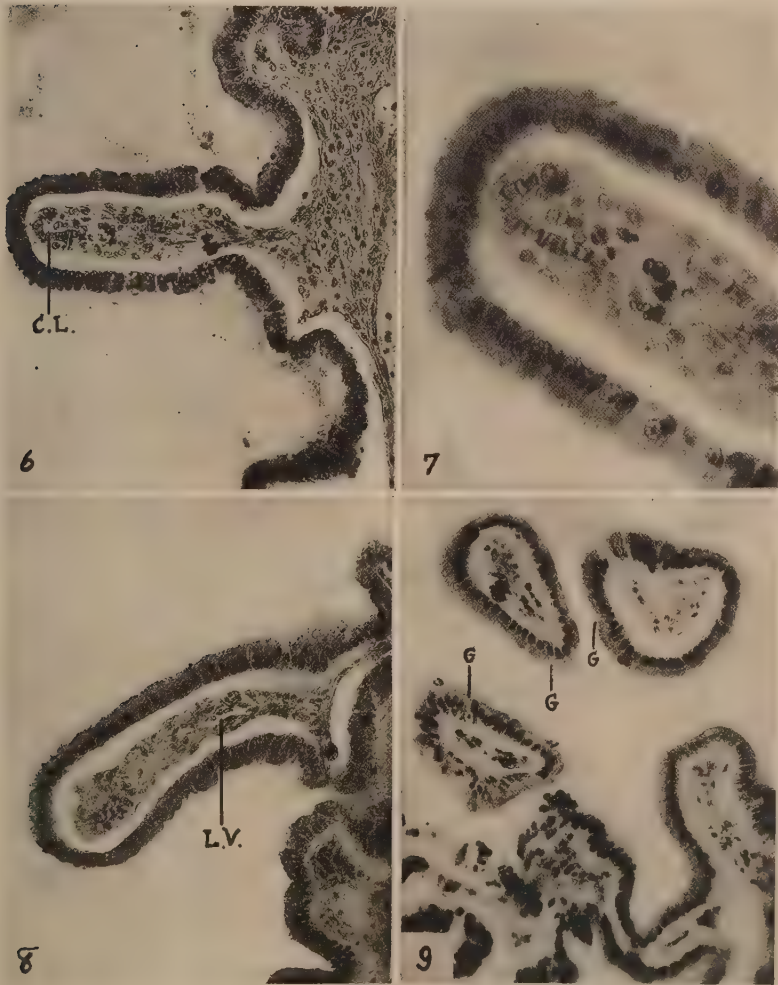


PLATE 3

EXPLANATION OF FIGURES

10 On the left is a section of the pancreatic bladder. Attached to it, a section of gall bladder is seen at the right of the photograph. $\times 17$. Note the difference in structure between the walls of the two vesicles.

11 Section of gall bladder attached to the pancreatic bladder in figure 10. $\times 53$.

12 Section of the pancreatic bladder, showing the secondary vesicle embedded within its wall. $\times 17$. This vesicle lies approximately 180° from the point of attachment of pancreatic bladder to gall bladder.

13 The secondary vesicle photographed from the same slide as in figure 12. $\times 53$. Note the similarity in structure to the section of gall bladder as seen in figure 11.

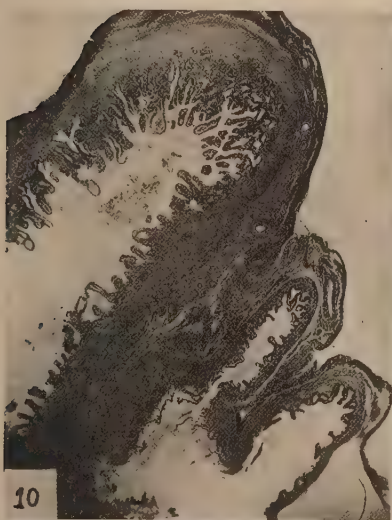
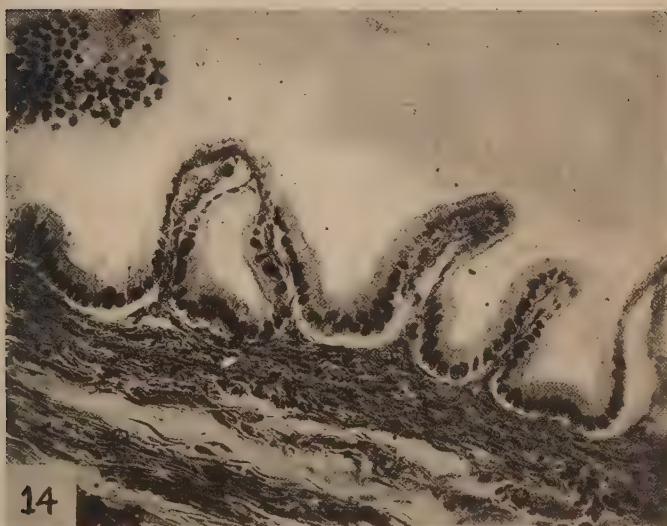


PLATE 4

EXPLANATION OF FIGURES

14 Transverse section of the wall of the secondary vesicle. $\times 300$. Note the plicate pattern of the mucous membrane and the absence of submucous glands, characteristic for normal gall bladder.

15 Section of the duodenum, showing the accessory pancreas which lay between the submucous and muscular layers. $\times 70$. At the left portions of villi and the submucous glands of the duodenum may be seen. At the right lies a portion of pancreatic tissue, showing lobules and interalveolar islets. Note the absence of interlobular ducts.



ON THE NATURE OF THE CAPSULE AND FRAMEWORK OF THE FAT-CORPUSCLE

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SIX FIGURES

The term fat-corpuscle as used in this paper refers to the fat-cell together with its enclosing and supporting fibrous membrane or capsule; the corpuscles being the characterizing components of adipose tissue. Adipose tissue is defined as an aggregation of fat-corpuscles held together by a coarse meshwork of intercorpuscular fibrous tissue, blood vessels, and nerve twigs. Thus this variety of tissue is structurally similar to other varieties, muscular, nervous, etc., in that its essential elements are supported by a fibrous framework. The paper does not attempt to deal with adipose tissue as a whole, but only with the fat-corpuscles.

The mature fat-corpuscle, in the general conception, consists of a mesenchymal cell differentiated for the storing of fat, the accumulating smaller fat-droplets fusing into larger until there results a single large globule of fat so distending the cell that the original cytoplasm and nucleus of the lipoblast is compressed to form the thin covering or 'signet-ring cell' about the globule. The capsule about the whole is seldom discussed, and a supportive framework permeating the globule of fat is not so much as intimated. In fact, the prevailing idea seems to be that the globule can have no framework within it. Hardesty ('05), dealing with the nature of the medullary sheaths of the nerve fibers, suggested that the medullary sheath of the nerve fiber may contain a supporting framework. After the technique employed, he pictures such a framework in the fully grown sheath; in the embryo fat-

droplets adhere to the axone along its course in the mesenchyme, first giving it a beaded appearance as described by Bardeen ('03); and Hardesty assumes that these smaller droplets fuse to form larger ones, which become large enough to surround the axone in places, and that as continuous fusion results in the continuous fatty sheath of the internode, some of the connective-tissue fibrils then forming in the vicinity become involved within the accumulating fat, others being packed off by it and condensed on its surface to form the neurilemma. In this explanation of the origin of the neurilemma, the sheath nuclei of the neurilemma represent the nuclei of the fibroblasts concerned with the origin of both the framework within the fat and the neurilemma or capsule about it. To quote Hardesty directly:

The seal-ring cells of the fetus and the sheath nuclei of the adult spinal cord represent 'cells' derived from the syncytium and whose activities result in the reticulated framework of the medullary sheath; that, wherever found, the protoplasm about the nucleus represents only the endoplasm which is being transformed into exoplasm which in its turn is transformed into the lamellated reticulum by a process similar to that described by Mall in the development of the fibrous connective tissue; and finally, that the origin of the framework and the origin of the sheath of Schwann of the peripheral fibers may be similar.

He states that the internode may exhibit one, more than one, or occasionally no sheath nucleus. He believes that if the accumulating myelin forming the fatty sheath exists in the form of an emulsion, it must be an emulsion which, in the fresh condition, is of such finely divided form as to be invisible. The nerve fibers themselves are sufficiently small for a mass of them to appear white by reflected light. Hardesty's work suggested that there might exist within the globule of fat-corpuscles a framework similar to that described for the medullary sheath.

Wasserman ('26) gives an idea as to the origin of the fat-corpuscle that is strikingly similar to Hardesty's idea concerning the origin of the myelin of the medullary sheath.

Wasserman asserts that the small fat-globules collect within the mesenchymal protoplasmic syncytium that is in close proximity to blood vessels. This syncytium contains within its meshes a fibrillated exoplasm. As the fat-particles consolidate to form large fat-drops, the protoplasmic syncytium immediately surrounding the contained fat becomes isolated so that a single corpuscle is formed. This rim of cytoplasm may, therefore, contain one, more than one, or no nucleus. At the same time, the fibrillated exoplasm is incorporated within the fat-organ as a medium of support. The fat-corpuscle, then, in some respects, resembles the internode of the medullary sheath. Wasserman does not, however, discuss a fibrous framework within the globule of the fat-corpuscle as described by Hardesty for the medullary sheath.

This bit of work was suggested to determine whether the older conception of the 'fat-cell' as a globule of fat limited by a peripheral rim of cytoplasm containing a nucleus may not be modified; that is, whether the fat-corpuscle, regardless of the process of its origin, may not contain within its body a supportive framework similar to the so-called neurokeratin of the myelin sheath. Hardesty ('05) describes this framework as a finely meshed, lamellated reticulum that is continuous with the outer and inner membranes of the medullary sheath. He claims that the meshes of the reticulated framework are occupied by myelin that, during life, without doubt exists in a finely divided form—an emulsion. In this finely divided fresh condition the myelin is distributed evenly throughout the sheath framework supporting it; in the post-mortem coarser globular form the normal arrangement of the framework is distorted by a continued coalescence of the smaller into larger globules, finally condensing the framework in places to form the purely postmortem Schmidt-Lantermann 'clefts.' Nemiloff ('10), using a precipitation method, likewise describes a supportive framework of much coarser filaments and heavier clefts, because of the metallic impregnation. He applies the old term 'neurokeratin' to the framework, but holds that it is continuous with the cytoplasmic

processes of the neurilemma cell. He also pictures (his fig. 5) vacuoles of varying sizes in the cytoplasmic structure. If the vacuoles are numerous, the cytoplasm appears foam-like; but if the vacuoles are sparse, there are seen thicker cytoplasmic partitions between them. He assumes that these vacuoles represent droplets of myelin, and that the varying granular, fibrillar, and vacuolized structure of the cytoplasm described by him corresponds to different stages of formation of myelin in the cytoplasm.

That the fat-corpuscle is confined by a membrane has long been known. Flemming ('71) describes the cell membrane of the serous fat-cell as a separate capsule that surrounds all parts of the cell. He claims that this membrane, which is absent in young fat cells, is not an integral part of the protoplasm, but a secondary cell product—either an excretion or changes of the periphery of the cell body comparable to the cuticle of certain epithelial cells. Frommann ('84) questions whether the membrane arises as a condensation of the peripheral protoplasm of the fat-cell or by a chemical change of the cytoplasm. He finally concludes that it arises as the result of a chemical change in the cytoplasm. He describes this change occurring in the original homogeneous surface of the cell as first appearing granular, then the granules in areas of the surface coalescing to form short, delicate fibrils, these areas spreading to involve the entire surface and later becoming a membrane homogeneous in appearance. This membrane, he states, is continuous with the peripheral layer of the cytoplasm of the fat-cell. Bell ('09) likewise describes the membrane of the fat-corpuscle, which he claims differs chemically from the protoplasm and from connective-tissue fibers, as differentiating from the peripheral layer of the cytoplasm of the cell. Schäfer ('24) also states that the membrane is a superficial differentiation of the protoplasm.

The text-books of Ranvier ('89), Böhm and von Davidoff-Huber, and Piersol limit the content of the fat-corpuscle by an envelope consisting of a transparent homogeneous membrane with a thin layer of cell protoplasm plastered on its

inner surface. Ranvier states that, after extraction of the fat-content by ether, this membrane appears in wrinkled form. In general, the texts of Schäfer, Ferguson, Keibel and Mall, Prenant, Bouin and Maillard, Krause, Lewis and Stöhr, Bailey and Miller, and Jordan present the limiting membrane or cell wall of the fat-corpusele as the protoplasmic rim of the cell pressed to the periphery as the accumulating fat-droplets fuse to form globules of increasing size.

Hammar ('95) denies the existence of a fat-cell membrane. Nemiloff ('06) states specifically that the fat-cell from *Acipenser ruthenus* possesses a wall, which he calls protoplasmic, that appears distinctly with the usual stains. In sections impregnated with silver as employed by Cajal, Nemiloff observes that, continuous with and from this protoplasmic wall, there passes into the interior of the corpusele numerous protoplasmic partitions or trabeculae (peculiar to *Acipenser ruthenus*) which are also continuous with the cytoplasm about the eccentrically placed nucleus. These trabeculae separate the individual fat-drops and likewise cleave the entire fat-cell into several parts. From this coarse-meshed ground net, the meshes of which correspond to larger fat-drops, there arises another extremely delicate network. The coarseness (his fig. 6) or fineness (his fig. 4) of this network varies with the degree of vacuolization of the protoplasmic partitions; that is, the finer the emulsification of the fat, the finer the network, etc. Under low power, this continuous system of protoplasmic trabeculae represents a network of fibers in the meshes of which are embedded fat-drops. He concludes, however, that in these protoplasmic trabeculae the cytoplasmic fibrils or sharply marked spongioplastic structure are not differentiated. Heidenhain ('11) interprets Nemiloff's findings in terms of a special type of vacuolar system of cytoplasmic arrangement.

Marchand ('19) considers the cell membrane of the fat-corpusele as a sort of cuticular structure, on the inner side of which lies the protoplasmic remains of the nucleus. He considers that this membrane is not of protoplasmic nature,

but is equivalent to the sarcolemma of muscle fibers and similar to the capsule of cartilage corpuscles. Even though his membrane assumes the same coloration as collagenous fibers, he emphasizes the fact that one cannot conclude that the two are of the same nature. He suggests, however, that it is not improbable that the behavior of the membranes varies with age and location, and that neither is it impossible for the membrane under certain circumstances to become collagenous. Chlopkow ('26) states that the sarcolemma of cardiac muscle is composed of two collagenous components, a basic homogeneous membrane and peripherally coursing bars of compact fibrillae. The majority of these peripheral bars course transversely (coinciding with the Z line); others course longitudinally. He also states that the sarcolemmas of adjacent muscle fibers are united by plates of connective tissues at their approximated surfaces.

Wasserman ('26) describes the tissue of a primitive fatlobule as composed of a syncytium in which fat storing takes place without the appearance of individual fat-forming cell. In his schematic drawing, figure 28, he illustrates the development of the fat-organ from a protoplasmic syncytium that contains in its meshes fibrillated exoplasmic membranes spread in all planes. He states that, as the small fat-droplets infiltrating the syncytium aggregate to form larger globules, there is a dispersion of the syncytium so that each larger globule becomes surrounded by a syncytial mass that contains an occasional nucleus. At the same time, the fibrillated exoplasmic membranes serve in the fat-organ as a medium of support. He claims that in reality there is no cell membrane, since neither the syncytial mass nor its exoplasmic reticulum can be termed as such.

In making this study of the fat-corpuscle, adipose tissue from numerous localities in man, dog, cat, rabbit, and ox was used. Some of the sections were treated by the Benda neuroglia method as employed by Huber ('01) and later by Hardesty ('05); others were made from pieces which after fixation were embedded, some in paraffin, some in celloidin, and

some in 50 per cent gelatin, sectioned at varying thicknesses and stained with various reagents: Weigert's, orcein, Mallory's connective-tissue stain, iron hematoxylin, hematoxylin eosin, and Heidenhain's chrome hematoxylin. Other sections of adipose tissue were prepared according to the technique used by Chlopkow ('26) in his study of the structure of the sarcolemma of cardiac muscle. Spreads of mesentery were treated with silver nitrate. Teased, spread, and frozen sections were likewise stained, and also teased preparations were treated with osmic fuchsin, osmic sublimate, and osmic sublimate-fuchsin.

Great difficulty was experienced in this laboratory in the embedding of adipose tissue. Dr. Julian Hawthorne, prior to this undertaking, experimented with paraffin and with castile soap as embedding media. He found that the hot paraffin melted the contained fat, allowing the corpuscles to become so collapsed that they were unsuited for study, and that the castile soap proved to be of too soft a consistency to give satisfactory results. So, when this study was begun, the paramount problem was to find an agent that would embed adipose tissue without extracting the contained fat, in order that the form and internal structure of the corpuscles could be preserved. All attempts were at first unsatisfactory. Finally, however, some blocks of tissue were successfully embedded in gelatin (50 per cent) shortly before its congealing upon cooling. Celloidin, as an embedding medium, gave but fair results for the study of the capsule of the corpuscle only. With the application of the method used by Chlopkow, paraffin embedding was highly satisfactory, because the structures desired were previously fixed and also impregnated before the application of the hot paraffin.

Sections of adipose tissue were also subjected to the action of pancreatin. Owing, however, to the unstable nature of the fat-content, it was impossible to retain the sections on the slides throughout the process of digestion. The sediments, when stained and examined, revealed fragmented connective tissue in which it was impossible to determine definite forms of the fat-corpuscles.

Of the stains employed upon the sections, 20 μ sections cut with the freezing microtome, and stained with Mallory's connective-tissue stain, were found most suitable for the study of the capsule. Sections of embedded material, stained with Mallory's, were also studied, as were spreads of mesentery treated with silver nitrate. Two of the methods used by Chlopkow proved best for the reticulum within the corpuscle. As used here, this procedure was as follows: Retain fresh blocks of material in Golgi fixing fluid for two days, then in 1 per cent silver-nitrate solution for two days. Dehydrate and embed in paraffin. Now proceed in one of two ways: 1) Treat sections with gold chloride to dissolve reduced silver. Stain with Mallory's. 2) Decolorize paraffin sections with freshly prepared mixture of twenty-five parts of a 20 per cent solution of sodium sulphate in distilled water and one part of a 20 per cent solution of potassium ferrous cyanide in distilled water. Stain with Heidenhain's iron hematoxylin followed by Mallory's connective-tissue stain.

As stained by Mallory's connective-tissue stain, the capsule assumes the same coloration as the intercorpuscular white fibrous support of the adipose tissue. It appears as a thin film of almost homogeneous texture, thought possibly to be a fine feltwork of white fibrous connective tissue. In many preparations this capsule is traversed by rib-like structures that seem to be a denser condensation of the supportive feltwork. These rib-like structures arise by gradually pulling away from compact lines of fibrillae situated like fence-posts in the angles formed by adjacent sides of the same and adjoining corpuscles, and extend practically parallel across the surface of the corpuscle to the next post, simulating rails of a fence and making the whole capsule in section to resemble a polyhedral rail pen (fig. 1). These ribs, however, do not necessarily appear to interdigitate, but rather rails in line with each other appear continuous at their common post of origin (fig. 1). Neither do they conform in all capsules to the same size and arrangement. While some of the rails retain practically the same diameter throughout their course across a

side of the pen, others exhibit areas of thickening or thinning (figs. 1 and 2). Neither can the ribs be followed at all times as distinct and individual structures, for they frequently split and rejoin, join an adjacent rib, or become lost in the substance of the membrane (figs. 1 and 2). Mallory's connective-tissue stain brings them out more strikingly than any of the other stains tried, though their presence is indicated by other stains.

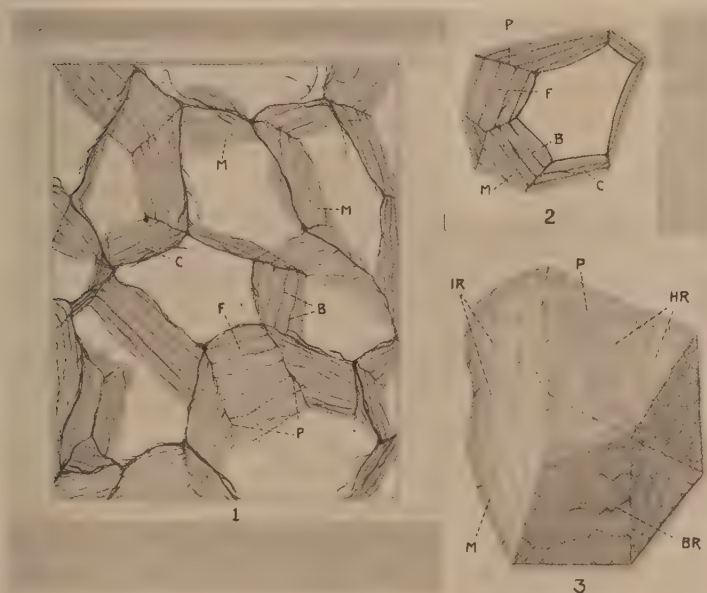


Fig. 1 Area from a section of human adipose tissue, showing polyhedral pen effect of capsule. Frozen section (20μ) stained with Mallory's connective-tissue stain. *M*, thin, apparently homogeneous membrane; *B*, bars of compact fibrillae contained within or upon *M*; *F*, collateral filament; *C*, cleft of rib *B*; *P*, post of compact fibrillae.

Fig. 2 Capsule of individual fat-corpuscle. Same as figure 1.

Fig. 3 Surface view of somewhat isolated fat-corpuscle, one side free, showing reticular pattern of capsule. Same technique as figure 1. *M*, thin, apparently homogeneous, membrane; *IR*, incomplete, widely spaced reticulum; *HR*, closely meshed, heavier reticulum; *BR*, reticulum simulating ribs *B* of figures 1 and 2; *P*, post of compact fibrillae.

Though most of the capsules comply with the heavy-ribbed effect described, all of them do not. Some of the capsules of smaller corpuscles and even some sides of the capsule of larger corpuscles that otherwise present the rib formation appear as a thin film, within or upon which is embedded many delicate, short fiber bundles. These bundles seem of the same structure as the heavy ribs described, but, instead of being parallel, they suggest an irregular reticulum, stress upon which has not been sufficient to produce the ribbed pattern. Absence of the ribbed appearance may be due to differences in stain technique or to age differences in the stages of the processes by which the large fat-corpuscles are formed.

In regions where the corpuscles are less densely packed, and even the sides subjected to less stress of large, somewhat densely aggregated corpuscles, the capsule appears composed of the same delicate felt-work carrying the fibrillar bundles arranged in reticular formation (fig. 3). These bundles exhibit a fuzzy nature and the reticulum formed by them is incomplete and widely spaced (fig. 3). In other capsules the reticulum becomes more pronounced, forming a closer-meshed reticular pattern of heavier bundles (fig. 3). In places this surface reticulum shows evidence of being pulled or drawn out into parallel lines (fig. 3), thus giving the ribbed arrangement previously observed, assumed to be produced by increased fat-content (increased stress). The reticulum grades into the larger compact bundles of fibrillae situated post-like in the angles formed by adjacent sides of the polyhedral capsule of the corpuscle (fig. 3). Some or all of the sides of other capsules show neither of the preceding patterns, the capsule in such cases being composed of only the delicate, apparently homogeneous, membrane. All of the isolated (not aggregated) round or oval fat-corpuscles, when viewed on the surface, are enclosed by a capsule that is apparently the same as the thin film or membrane that was depicted as carrying the ribbed and reticular patterns.

Aside from the structures of the capsule observed, an occasional corpuscle discloses a beautiful reticular formation,

the location of which in the sections is difficult to determine; that is, whether it is part of the capsule or in the body of the corpuscle. The general picture, however, is strongly suggestive of the presence of a fine-meshed reticulum within the corpuscle, serving as a supportive framework for the fat-content, the framework being of such a delicate nature that it is broken and usually washed out with the extraction of the fat.

Sections prepared by the method described by Chlopkow seem to preserve the presence of this framework, and, being a precipitation method (silver nitrate and gold chloride), it exaggerates the filaments of the framework so that it appears quite pronounced and decidedly within the corpuscles. At the periphery of the sections (the block of tissue) where the precipitation is extremely heavy, the detail structure is obscured, as a rule, while near the center of the section the framework is absent or very faintly evident, due perhaps to the poorer fixation and slower infiltration in this region. Between these extremes, but by no means limited to it, is an area in which practically all of the corpuscles display a reticulated intra-corpuscular framework that exhibits meshes of various sizes and shapes. In the majority of the corpuscles the reticulum appears as bundles of delicate, fuzzy fibrils that extend in all planes, the bundles and their fibrils appear thread-like and not as sections of lamellae (fig. 4). The small irregularly shaped meshes of this reticulum are intimately occupied throughout by the fat. If this contained fat is in the form of globules—that is, an emulsion—the irregular shape of the meshes suggests either that the droplets are of necessity greatly distorted by mechanical stress or postmortem changes in order to occupy the meshes of such shapes, or the meshes are distorted after removal of the droplets. It is not impossible, however, that the fat in the normal fresh condition may exist in a condition so finely emulsified as to form apparently one mass which is supported by the reticulum permeating it throughout the body of the corpuscle. If such is true, the larger globules, resulting from coalescence of

smaller, could be postmortem phenomena. On the other hand, if the load of the large fat-corpuscle consists of one large droplet, the reticular framework may be dispersed throughout the droplet. Whether the threads of the reticulum consist of the cytoplasm of the original mesenchymal element dispersed into filaments by the accumulating fat-droplets or are fibrous products of the protoplasm rather than a part of its structure has not yet been definitely determined. Being continuous with the capsule (fig. 4), they may be assumed of the origin and nature of the capsule.

The framework in some corpuscles exhibits in certain parts, in addition to the fine-meshed reticulum, a densely stained, coarser-meshed reticulum whose meshes possibly represent the packing off of the finer-meshed framework by the continued coalescence of smaller fat-globules. The coarser threads of such coarser meshes, though doubtless composed of condensations of finer threads, appear homogeneous after the precipitation method of staining used. One is impressed with the idea that even these larger spaces are not always entirely free from the supportive framework, since from the condensed threads numerous finer threads extend into the spaces as though they have been incorporated by the larger globule during its formation. Quite often clear spaces of varying size, free of the reticulum, appear in the body of the corpuscle (fig. 4). These spaces are thought to be due to the reticulum having been washed out during manipulation of the material, and may be due to poor fixation. They do not suggest that they represent the position of removed large droplets. In still other corpuscles the coarser reticulum which usually stains heavy is seen retracted from the capsule in whole or in part, and condensed toward the center (fig. 5) or collapsed toward the side of the corpuscle. Such cases are considered shrinkage artifacts. Their irregular meshes appear bridged by a fine framework of smaller meshes likewise distorted. These corpuscles with the collapsed, coarser-threaded and coarser-meshed reticulum are found in the zone of the block next its densely precipitated and obscured pe-

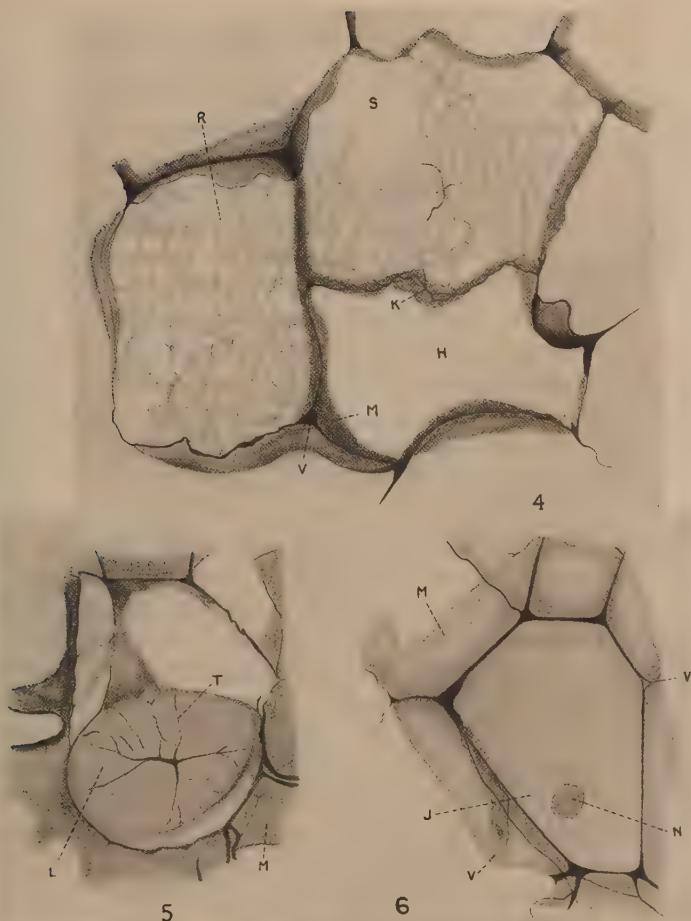


Fig. 4 Fat-corpuscle from adipose tissue of ox, showing reticular framework within the body of the corpuscle. Chlopkow's method, designated *I* here. *R*, framework composed of many delicate, short fibril bundles; *K*, framework joining capsule of corpuscle; *S*, clear space within corpuscle; *H*, corpuscle entirely free of reticulum, probably washed out in treatment; *M*, thin, apparently homogeneous membrane of capsule; *V*, blood vessel.

Fig. 5 Fat-corpuscle, showing retraction of reticulum, doubtless shrinkage effect. Same method as figure 4. *T*, condensation of framework; *L*, delicate framework uniting concentrated lines *AF*; *M*, thin, apparently homogeneous membrane of capsule.

Fig. 6 Fat-corpuscle, showing a round-meshed reticulum within the fat-corpuscle, immediately within the capsule. Same method as figure 4. *J*, round-meshed reticulum; *N*, probable nucleus; *M*, thin, apparently homogeneous membrane of capsule; *V*, blood vessels.

riphery, where the fat has been more rapidly extracted and the reticulum probably more subject to results of treatment, the corpuscles with the more evenly distributed framework being in the next zone within, while, if the block be large, the corpuscle of its center shows no reticulum at all, probably due to the poor fixation of this part of the fat-carrying fresh material.

In addition to the various appearances presented by the type of framework described above, there may be observed a second type of reticulum, situated just within the capsule and extending varying small distances toward the center of the corpuscle. The threads of this reticulum do not suggest the finely fibrillated nature observed in the irregular-meshed framework. Its meshes are circular in sections, doubtless spherical in toto, and, though small in general, are subject to very wide variations in size (fig. 6). Its threads are the shape of the spaces between the circles or spheres, being irregular in thickness and triangular-shaped in parts between closely aggregated circles. A spherical structure, apparently a nucleus, but not conclusively so, since a nuclear stain was not used, is occasionally seen situated in the periphery of this round-meshed reticulum (fig. 6). This reticulum with the probable nucleus is interpreted as representing the cytoplasm and nucleus of the original fat-storing protoplasm compressed to the periphery and appearing in section as the 'signet-ring cell' of the usual descriptions of fat, the very varying circular meshes representing the cytoplasm vacuolated by small fat-globules. Therefore, the boundaries of the meshes of the reticulum (the globules) are but cytoplasm filling the interglobular spaces, and are membranes instead of threads. At one place only this round-meshed reticulum was seen grading into and giving way to the finely fibrillated framework first described within the corpuscle. Occasionally corpuscles reveal both the fibrillated and the round-meshed reticulum. In such cases the round-meshed framework is situated peripherally and at the surface of the corpuscle, while the irregular-meshed framework is situated deeper in the body of the fat-corpuscle.

Since the preparations used do not differentiate the exact nature of structures described, it is impossible at this time to make definite statements concerning their histological components. However, in view of the findings reported here, it is assumed that these various reticular patterns represent stages in the accumulation of fat within the corpuscle. The peripherally placed reticulum of small but irregular-sized round meshes probably represents the last part of the cytoplasm of the fat-corpuscle to accumulate fat-droplets. The cytoplasm enclosing these small fat-droplets would present the vacuolated or round-meshed reticular appearance after extraction of the contained fat. As the small droplets coalesce to form globules of increasing size, this cytoplasm would become more dispersed. In becoming so, it is suggested that it is converted into the fibrillar products that form the sponge-like framework described above and more commonly present throughout the corpuscle.

The capsules of the fat-corpuscles appear joined with the framework; the latter, however, does not exhibit evidence of forming its related capsule by a concentration peripherally of its constituents. The capsules, when prepared according to Chlopkow's technique, do not present the definite structure observed in sections stained only with Mallory's connective-tissue stain. This by no means implies that the ribbed or reticular patterns are entirely absent, but simply that they may not be so differentially stained or so numerous as in the other sections. This difference may be due to either a difference in the preparation of the sections or to a difference in the material used.

In view of the above observations, it is suggested that the capsule of the fat-corpuscle under stress is somewhat comparable to the structure of the sarcolemma reported by Chlopkow. The envelope in both cases exhibits two parts, a thin, apparently homogeneous membrane, within or upon which is embedded delicate fibrillae arranged in bundles (Chlopkow's fig. 3 and figs. 1 and 2 of this paper). At this time, however, it is impossible to definitely assert that the capsule of the

fat-corpusele is composed of collagenous fibers, which Chlopkow concludes from both components of the sarcolemma. Marchand, prior to Chlopkow's very interesting work, likens the cell membrane of the fat-corpusele to the sarcolemma of cardiac muscle fibers. A comparison of Chlopkow's figure 3 and figure 1 of this paper substantiates the similarity, suggested by Marchand, existing between the capsule of the fat-corpusele and the sarcolemma of cardiac muscle fibers. Marchand, however, does not ascribe a collagenous nature to the capsule, but states that the membrane and collagenous fibers assume the same coloration as reported here, and that it is not impossible for the membrane to become collagenous under certain circumstances. The observation of Marchand as well as Flemming and Frommann that 'membranes' of fat-corpuseles vary under different circumstances are confirmed in this study.

The text-books of Ranvier, Böhm and von Davidoff-Huber, and Piersol give the idea of the envelope of the fat-corpusele having two components, a thin homogeneous membrane with a layer of cell protoplasm spread on the inner surface of the membrane. Their descriptions must refer to the thin, apparently homogeneous membrane (free from reticulum or ribs) and the round-meshed, probably cytoplasmic, reticulum just within the capsule here described. Ranvier also states that, after extraction of fat, this membrane appears in wrinkled form. The possibility that the ribs featured here as a component of the capsule may be merely folds or wrinkles produced mechanically is suggested. However, after careful study of the nature of the ribs or ridges, it seems that, while some of them may be artificially produced, certainly most of them are not, for they appear too constantly and too uniformly to be due to mechanical effects. Furthermore, Ranvier's observations were based on a study of material from which fat was extracted by ether, thus allowing the capsules to shrink, while for this study some of the sections showing the ribs were from blocks of tissue fixed in formalin which seems to produce a slight swelling of the fat-content. The

observations of Schäfer and others who present the membrane as a rim of cytoplasm are confirmed by this work, if one interprets the cytoplasm exhibiting the round-meshed reticulum described here as the cell membrane.

Wasserman gives the idea that neither the syncytial mass nor the exoplasmic product of the syncytium between the fat-globules can be termed cell membrane. This study was begun some time previous to Wasserman's publication, but, in terms of his work, the capsules with their varying patterns (incident with increased stress) here described probably correspond to his fibrillated exoplasmic membranes that are enclosed in the fat-organ as a medium of support. His syncytial mass pressed to the periphery of the globules by increased fat-content possibly represents the cytoplasm that shows here a round-meshed reticulum. He, however, describes nothing that corresponds to the framework observed in the corpuscle. Nemiloff shows in his figures 4 and 6 structures that are almost analogous to the observations reported here. He, however, observed these protoplasmic partitions within the corpuscle, probably the reticulated framework portrayed in this study, only in the *Acipenser ruthenus*. On the contrary, our fat-corpuscles, from human and ox, subjected to Chlopkow's technique, displayed definitely the supportive framework, and almost all of the material stained with Mallory's suggests it. Nemiloff also pictures the endoplasm about the nucleus as continuous with these protoplasmic partitions, which in turn are continuous with the protoplasmic wall. In this study also the reticulated framework was continuous with the round-meshed reticulum and joined one of the types of capsules here depicted. The round-meshed reticulum seemed to lose its identity in the irregular-meshed reticulum with which it is continuous. Nemiloff states that the protoplasmic partitions separate the fat-drops and thus simultaneously cleave the cell content into several parts. His description of the incorporated fat conforms to the assumption here that, as finer globules of fat coalesce to form larger, probably post-mortem, the natural arrangement of the framework is dis-

torted. Or probably, as previously stated, fat-corpuseles in various stages of formation present different structural features. Even though the protoplasmic partitions described by Nemiloff suggest being composed of a fibrillated network, he concludes that it is impossible to differentiate protoplasmic fibrils and sharply marked spongioplastic structure by the method used in preparing the section. Likewise, study here fails to discern the exact nature of the framework. It is probably dispersed cytoplasm or fibrillated protoplasm, the fibrillae being a product of, and not a part of, the protoplasm. Furthermore, Nemiloff pictures (fig. 5), under his discussion of medullated nerves, a vacuolization of cytoplasmic processes that resembles the round-meshed reticulum described in this paper (fig. 6). He assumes that the granular, fibrillar, and vacuolized structure of these cytoplasmic processes correspond to different stages in the formation of myelin just as the various reticular patterns and capsular structures are assumed here to represent different stages in the accumulation of fat within the fat-corpusele.

For the drawings, I wish to thank Messrs. R. L. Hargrave, T. B. McKneely, and H. A. Thomas, students in the School of Medicine.

SUMMARY

1. The capsule of the fat-corpusele consists of *a*) a very delicate membrane of almost homogeneous texture, possibly a fine feltwork of white fibrous connective tissue, within or upon which is embedded, *b*) delicate bundles of collagenous fibrillae. These bundles of fibrillae vary in their arrangement, depending probably upon the size of the fat-corpusele and the amount of stress to which the capsule is subjected. In small corpuseles the bundles are not differentiated from the delicate membrane or background that supports them. As progressively larger corpuseles are studied, the fibrillae are observed in reticular formation. In some capsules this reticulum is incomplete and widely spaced; in others it becomes more pronounced, forming a close-meshed reticular pattern

of heavier bundles. The reticulum then covers most of the surfaces of the now polyhedral corpuscles and grades into larger bundles of compact fibrillae situated like fence posts in the angles formed by adjacent sides of the polyhedron. As the capsules become subjected to greater stress (increased fat-content), the reticulum appears drawn out, forming parallel, rib-like bundles that arise from the 'posts,' common not only to adjacent sides of the same corpuscle, but also to adjacent sides of adjoining corpuscles. These ribs extend across the surface of the corpuscle, simulating rails of a fence, which, however, frequently split and rejoin, join an adjacent rib, or become lost in the substance of the membrane.

2. Situated just within the variously patterned capsules and extending varying short distances into the body of the fat-corpuscles is a round-meshed reticulum that appears as a thin membrane with openings (vacuoles) in it. Occasionally a spherical structure, probably a nucleus, is associated with this reticulum. Within the body of many fat-corpuscles and continuous with their capsules is a delicate reticulum that appears as bundles of fine, fuzzy fibrils that extend in all planes, and form an irregular-sized and -shaped mesh-work that supports the fat. This fibrillated framework is at times continuous with the peripherally situated round-meshed reticulum.

3. While it is impossible at present to make definite assertions concerning the exact nature of the two general types of framework within the corpuscle, it might be assumed that the round-meshed reticulum represents the last cytoplasm to collect fat-droplets and that the fibrillated framework represents the same cytoplasm dispersed by increasing fat coalescence, being finally converted into its fibrillar product. Neither is it possible to state the exact nature of the capsule of the fat-corpuscle. It may be presumed, however, that both components of the capsule, the thin, apparently homogeneous, membrane and the bundles of compact fibrillae, are collagenous.

LITERATURE CITED

- BELL, E. T. 1909 On the histogenesis of the adipose tissue of the ox. *Am. Jour. Anat.*, vol. 9.
- CHLOPKOW, A. 1926 Zur Frage über die Struktur des Herzmuskel-sarkolemmas der Säugetiere. *Anat. Anzeig.*, Bd. 61, Oct. 11.
- FLEMMING, W. 1876 Beiträge zur Anatomie und Physiologie des Bindegewebes. *Arch. für mikros. Anat.*, Bd. 12.
- 1871 Weitere Mittheilungen. Zur Physiologie der Fettzelle. Schultze, *Archiv. für mikros. Anat.*, Bd. 7.
- 1871 Ueber Bildung und Rückbildung der Fettzelle im Bindegewebe usw. Schultze, *Archiv. für mikros. Anat.*, Bd. 7.
- FROMMANN, C. 1884 Ueber die Struktur der Fettzellen und ihrer Membran. *Naturwissenschaft*, Bd. 17.
- HAMMAR, J. A. 1895 Zur Kenntnis des Fettgewebes. Schultze, *Archiv. für mikros. Anat.*, Bd. 45.
- HARDESTY, IRVING 1905 On the occurrence of sheath cells and the nature of the axone sheaths in the central nervous system. *Am. Jour. Anat.*, vol. 4, no. 3, May 25.
- HEIDENHAIN, M. 1911 Plasma und Zelle.
- HUBER, G. C. 1901 Studies on the neuroglia. *Am. Jour. Anat.*, vol. 1, no. 1.
- LEWIS, FREDERIC T. 1925 A further study of the polyhedral shapes of cells. *Am. Acad. of Arts and Science*, December.
- MARCHAND, FELIX 1919-1920 Über die Veränderungen des Fettgewebes nach der Transplantation in einem Gehirndefekt mit Berücksichtigung der Regeneration desselben und der kleinzelligen Infiltration des Bindegewebes. *Beiträge zur Pathologischen Anat. und zur Allgemeiner Pathologie*, Bd. 66.
- NEMILOFF, ANTON VON 1906 Frage über den Bau der Fettzellen bei *Acipenser ruthenus*. *Anat. Anzeig.*, Bd. 28.
- 1910-1911 Über die Beziehung der sog. 'Zellen der Schwannschen Scheide' zum Myelin in den Nervenfasern von Säugetieren. *Archiv. f. mikrosk. Anatomie und Entwicklungsgeschichte*, Bd. 76.
- PRENANT, BOUIN, ET MAILLARD 1911 *Traite d'Histologie*.
- RANVIER, L. 1889 *Traite Technique d'Histologie*.
- WASSERMAN, F. 1926 Die Fettorgane des Menschen. *Zeitschrift für Zellforschung und mikrosk. Anatomie*, Bd. 3.

A DESCRIPTION OF A SIMPLE LABORATORY APPARATUS FOR OBTAINING ACCURATE TRACINGS OF OBJECTS

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ONE FIGURE

During the course of investigations the necessity continually arises of the securing of more or less faithful pictures of anatomical structures. The securing of such pictures is usually an arduous task and is almost insuperable to those who have little skill in free-hand drawing. Even where such skill exists the personal factor may lead to distortion of structures.

These difficulties may not arise in laboratories where ample funds are available for the purpose of elaborate instruments, such as a dioptograph or a macroscopic camera lucida, but even with these instruments the copying of large objects frequently presents formidable difficulties, and distortion is not easy to avoid. In smaller laboratories, where such facilities do not exist, it is always possible to secure pictures as faithful, or even more faithful than may be obtained with such expensive instruments, by the simple apparatus herein described. I have, therefore, been requested by Professor Dart to write an account of the apparatus which I have set up for use in the department of anatomy here, and which we have used extensively and successfully and in preference to the other instruments, although they are available in the department, especially where rapid drawings are required.

The apparatus, which can be assembled in about five minutes and of which a dozen can be prepared in a very

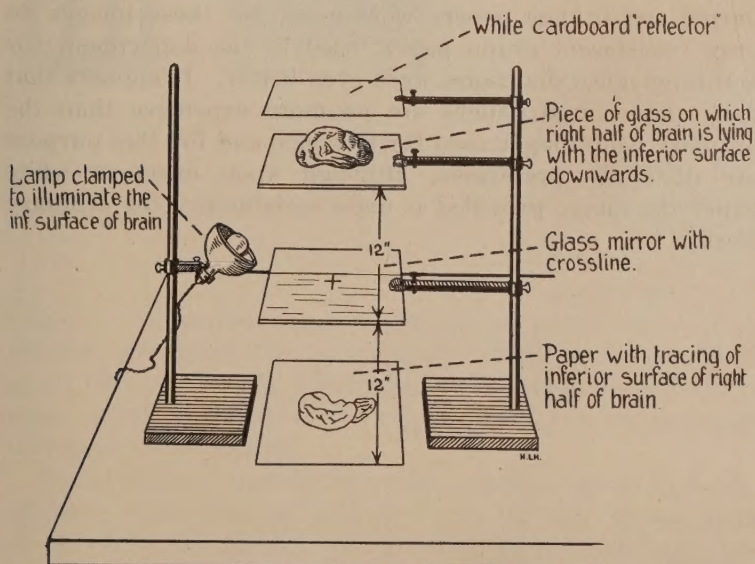
brief time and placed at the disposal of a whole class of students, consists merely of two sheets of ordinary glass (we use here discarded full-plate camera-glass sheets from which the gelatin negatives have been removed by washing), two retort stands with clamps, and an ordinary laboratory electric light. These are arranged as shown in the accompanying diagram.

Above a piece of paper on a table are clamped the two sheets of glass, one above the other, so that the distance between the lower glass and the table is the same as that between the upper and lower sheets of glass.

The object—in the case of the diagram, the right half of a brain lying with its inferior surface downward—is supported on the upper glass. The lower glass acts as a mirror, the image of the object being projected onto the paper below. This image is a perfect mirror image and presents no distortion except that which is due to imperfections in the glass itself, but this distortion is negligible. The image should be drawn on paper sufficiently thin to be reversed, so that the true image may be traced through onto the other side.

It is clear that the nearer the sheets of glass are to each other and to the table, the more clearly will the image appear, but sufficient space must be allowed between the sheets of glass and the table to admit, in the lower space, the hand and pencil of the observer, and in the space between the sheets of glass, the lamp which illuminates the object. Twelve inches in each case was found to be a convenient distance. The lamp is clamped onto a retort stand and so arranged that the light falls on the object only (fig. 1), because the more contrast there is between the illuminated object and the darker paper below, the more distinctly will the image appear. The lamp will, in the course of the tracing, need to be shifted from one position to another, so as to light up each side of the object in turn, as the drawing proceeds. This could be avoided, of course, by having several lights directed onto the object from different positions.

In order to secure an absolutely accurate drawing of the reflected object, the position of the observer must of necessity always be the same for the same tracing, since from every other position a slightly different image of the object is seen. To insure this accuracy, a mark is made on the lower glass—a cross-line drawn with India ink on the glass answers the purpose—and the observer keeps the cross fixed on any particular point throughout the procedure of tracing.



Diagrammatic sketch of Apparatus.

Figure 1

The image can be traced with perfect ease and clarity, and, provided that the light is strong, the tracing can be done in the ordinary light of the room, although the greater the contrast between the darkness of the surface on which the image is traced and the illumination of the object, the better will be the image. This end may be attained by darkening the room, but a more practicable method, as was

pointed out to me by Messrs. Wells and Thomas, in this department, is to clamp a piece of white cardboard horizontally above the object; this acts as a reflector and aids in the intensity of illumination, which factor seems to be the principal one in obtaining good results. Another important factor in obtaining a good and unblurred image is to use a dark paper for tracing. This, as stated, enhances the contrast between the illuminated object and the surface onto which the image is projected. The images on thin-matt purple and orange papers were good, but those images on gray translucent graph paper, used in the department for anthropological diagrams, were even better. It appears that dark and colored papers are no more expensive than the ordinary white paper used for tracings, and for this purpose are distinctly preferable, although upon ordinary white paper the image provided is quite satisfactory for ordinary work.